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RESEARCH ARTICLE | *Translational Research in Acute Lung Injury and Pulmonary Fibrosis*

Profiling of ARDS pulmonary edema fluid identifies a metabolically distinct subset

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Rogers AJ, Contrepois K, Wu M, Zheng M, Peltz G, Ware LB, Matthay MA. Profiling of ARDS pulmonary edema fluid identifies a metabolically distinct subset. *Am J Physiol Lung Cell Mol Physiol* 312: L703–L709, 2017. First published March 3, 2017; doi:10.1152/ajplung.00438.2016.—There is considerable biological and physiological heterogeneity among patients who meet standard clinical criteria for acute respiratory distress syndrome (ARDS). In this study, we tested the hypothesis that there exists a subgroup of ARDS patients who exhibit a metabolically distinct profile. We examined undiluted pulmonary edema fluid obtained at the time of endotracheal intubation from 16 clinically phenotyped ARDS patients and 13 control patients with hydrostatic pulmonary edema. Nontargeted metabolic profiling was carried out on the undiluted edema fluid. Univariate and multivariate statistical analyses including principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were conducted to find discriminant metabolites. Seven-hundred and sixty unique metabolites were identified in the pulmonary edema fluid of these 29 patients. We found that a subset of ARDS patients (6/16, 38%) presented a distinct metabolic profile with the overrepresentation of 235 metabolites compared with edema fluid from the other 10 ARDS patients, whose edema fluid metabolic profile was indistinguishable from those of the 13 control patients with hydrostatic edema. This "high metabolite" endotype was characterized by higher concentrations of metabolites belonging to all of the main metabolic classes including lipids, amino acids, and carbohydrates. This distinct group with high metabolite levels in the edema fluid was also associated with a higher mortality rate. Thus metabolic profiling of the edema fluid of ARDS patients supports the hypothesis that there is considerable biological heterogeneity among ARDS patients who meet standard clinical and physiological criteria for ARDS.

ARDS; metabolomics; endotype; pulmonary edema; phenotype

ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) remains a major medical problem in critical care, with recent estimates that 10% of ventilated patients develop ARDS and mortality rates as high as 40% for patients with severe ARDS (2). Despite more than 50 therapeutic clinical trials to date in ARDS, a dedicated drug to treat ARDS is still lacking, and mortality remains high (10).

A major barrier to new drug development in ARDS is heterogeneity of the syndrome, with diverse etiologies of lung injury (sepsis, pneumonia, trauma, and transfusions to name only a few) contributing to a common phenotype but potentially many different underlying pathophysiologies (12). Calfee et al. (4) recently reported that two ARDS cohorts [the ARMA study of low tidal volume and the ALVEOLI study of positive end-expiratory pressure (PEEP) titration] are each best characterized as two distinct classes. In both cohorts, one-third of the patients were classified as hyperinflammatory, with higher levels of inflammatory biomarkers and an increased need for vasopressors. Importantly, class status could not be defined by simply the ARDS risk factor or any routinely measured clinical variable. Class status also mattered in the therapeutic application of PEEP, with a significant interaction term for treatment effect of higher PEEP. Hyperinflammatory patients were less likely to die when randomized to higher PEEP. By contrast, patients with a less inflamed phenotype were less likely to die when randomized to lower PEEP. Similarly, in the ARDS Network Fluid and Catheter Treatment Trial (FACTT) of fluid management strategy, randomization to treatment with a conservative fluid strategy improved mortality in the hyperinflammatory subgroup, while a liberal fluid strategy improved mortality in the hypoinflammatory subjects (7).

In the current study, we examined the metabolic phenotype of undiluted pulmonary edema fluid from patients undergoing intubation and mechanical ventilation for acute hypoxemic respiratory failure. We have previously demonstrated that undiluted pulmonary edema fluid sampled at the time of intubation holds diagnostic and prognostic importance in ARDS (19, 20). ARDS edema fluid is characterized by a high protein content, and the edema fluid-to-plasma protein ratio can be used to discriminate ARDS from hydrostatic pulmonary edema (area under the curve of 0.8 separating hydrostatic vs ARDS fluid at a cut-off of 0.65) (19). The rate of protein clearance from sequential sampling of edema fluid also has prognostic importance in ARDS, with patients who fail to begin clearing

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edema within the first 6 h of intubation at higher risk of intensive care unit (ICU) death (20) and also a direct relationship between vasopressor use and impaired alveolar fluid clearance in patients with sepsis (23).

We hypothesized that pulmonary edema fluid metabolomics could identify a distinct class of ARDS patients. In this study, we carried out nontargeted metabolic profiling of undiluted edema fluid from 29 patients with pulmonary edema, including 16 with ARDS and 13 with hydrostatic edema, as phenotyped by two physician experts (M. A. Matthay and L. B. Ware). As hypothesized, within the ARDS cohort, metabolomic profiles of six subjects (~1/3 of patients) differed markedly, with upregulation across numerous chemical classes, while the other ARDS patients were not distinguishable from hydrostatic edema. These findings highlight both the likely presence of a hypermetabolic subtype of ARDS and the potential for metabolomics to identify novel mechanisms in ARDS pathobiology (14).

METHODS

Population. Patients studied in this analysis were derived from a cohort that has been described previously (19, 20). Briefly, patients with acute pulmonary edema who required endotracheal intubation for acute respiratory failure were enrolled between 1981 and 2007 at the University of California San Francisco Moffitt-Long Hospital and San Francisco General Hospital with institutional review board approval.

Subjects were clinically phenotyped by two expert reviewers at the time of discharge from the hospital, using all available clinical data including history, physical exam, laboratory, invasive monitoring data, autopsy results, physician summaries, and review of chest radiographs. The cause of acute pulmonary edema was classified as hydrostatic pulmonary edema or acute lung injury (with Pa_{O_2}/F_{IO_2} <300 mmHg) according to the American-European Consensus Conference definition (3) or indeterminate. Patients with acute lung injury are referred to as having ARDS, in keeping with the recent Berlin definition of ARDS (8). Clinical characterization was independent of edema fluid characterization and metabolic profiling.

Subjects selected for metabolic profiling included those with sufficient edema fluid available for testing (>500 μl) who were categorized as ARDS or hydrostatic edema by expert review (indeterminate cases were not included). To further reduce the chance of misclassification between ARDS and hydrostatic edema, we selected only subjects whose edema fluid-to-plasma protein ratio was concordant with clinical phenotyping if available (i.e., ≥0.65 for acute lung injury, <0.65 for hydrostatic edema).

Metabolic profiling strategy. Edema samples were obtained within 4 h of intubation and stored at −80°C. Aliquots (150 μl) were shipped to Metabolon (Durham, NC) for analysis on their nontargeted metabolome platform. Briefly, proteins were precipitated with methanol, and the resulting extracts were analyzed with four complementary methods: ultrahigh-performance liquid chromatography/tandem mass spectrometry (UHLC/MS/MS2) for basic species, UHLC/MS/MS2 optimized for acidic species, and UHLC/MS/MS2 for lipids. Data analysis was performed with Metabolon's software that includes peak peaking, retention time alignment, quantification, data curation, and normalization. Peaks were identified by matching against an in-house library of authentic standards and routinely detected unknown compounds. Additionally, each of the individual samples was combined to create a quality control sample to assess technical and process variability.

Data processing and statistical analyses. All analysis was performed using Metaboanalyst 3.0 and R v 3.0.1 (22). Metabolites without variability across samples removed from further analysis. Metabolic data were $\log_2$ transformed and auto scaled before statistical analysis (18). Individual metabolites were tested using the univariate Wilcoxon's rank sum test, with values of fold change of >2 and $P < 0.10$ considered significant. We performed hierarchical clustering using Euclidean distance in the hclust package in R.

Metabolomics data were further examined using multivariate analysis methods principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) (21). PCA reduces the dimensionality of the data in an unbiased and unsupervised fashion to visualize the structure of the data (i.e., cluster of samples, outliers). In contrast, PLS-DA is a supervised method that maximizes the differences between groups and is used to reveal discriminant metabolites. To ensure that between-group differences are robust, the q^2 value with leave one out partitioning must be at least 0.6 to be considered significant.

All metabolites that met the univariate significance threshold ($P < 0.1$ and fold change >2), with a confirmed Human Metabolome Database (HMDB) ID, were assessed for pathway overrepresentation using the hypergeometric test in the Pathway Analysis module in metaboAnalyst.ca. Node importance was assigned using relative betweenness centrality. Pathways with a false discovery rate (FDR) of $P < 0.05$ were considered significant.

RESULTS

Clinical characteristics. Pulmonary edema fluid from 16 subjects with ARDS and 13 subjects with hydrostatic edema was available for profiling. These samples were selected as phenotypically certain based on both clinical phenotyping by two experts (M. A. Matthay and L. B. Ware) and concordant

Table 1. *Demographics of 16 patients with ARDS vs. 13 patients with hydrostatic edema*

Clinical Characteristics	ARDS (n = 16)	Hydrostatic Edema (n = 13)	P Value
Age	42 [36.5, 49]	53 [34, 66]	0.33
Sex, %men	50%	62%	0.71
Vasopressor use	75%	46%	0.15
Mortality	44%	15%	0.13
Edema fluid:plasma protein ratio	0.84 [0.76, 0.96]	0.44 [0.41, 0.54]	1×10^{-5}
Primary diagnosis			4×10^{-5}
Type (no. of cases)	Pneumonia (4), sepsis (4), anaphylaxis (2), aspiration (1), TRALI (2), fulminant hepatic failure (1), reperfusion edema (1), tumor lysis (1)	Volume overload/hydrostatic edema (5), myocardial infarction/ischemia (2), cardiac arrest (1), postobstructive (2), cardiogenic shock (1), TACO (1), neurogenic (1)	

Median [interquartile range (IQR)], with P value by Wilcoxon's rank sum for continuous variables and Fisher's exact for categorical variables, is shown. ARDS, acute respiratory distress syndrome; TACO, transfusion-associated cardiac overload; TRALI, transfusion-associated lung injury; Sepsis, nonpulmonary source of sepsis.

edema fluid-to-plasma protein ratios. Samples with uncertain clinical phenotyping (clinical uncertainty of hydrostatic edema vs ARDS; $n = 3$) and those with discordant edema-fluid-to-plasma protein ratios ($n = 6$) were not selected for profiling. Clinical characteristics of the 29 subjects are provided in Table 1. These cohorts were very different clinically, with more sepsis, presence of ARDS risk factors, and a much higher mortality in subjects with ARDS and more heart disease in subjects with hydrostatic edema.

Identification of a high metabolite edema fluid subgroup of ARDS. We used the Metabolon broad spectrum platform for nontargeted profiling, which can identify more than 3,700 known human plasma metabolites. There were 760 metabolites identified in pulmonary edema fluid samples; 749 of these varied across samples. Hierarchical clustering showed that ARDS patients did not cluster separately from hydrostatic edema with the exception of a subset of 6 patients with ARDS (Fig. 1). These six patients could be clearly separated from the remaining 10 ARDS subjects and 13 subjects who were clinically phenotyped as hydrostatic edema on PCA and PLS-DA plots (Fig. 2). PLS-DA separation was stable with leave one out cross validation, with $q^2 > 0.6$.

The clinical characteristics of these 6 ARDS subjects compared with the other 10 subjects are shown in Table 2. This group is notable for including a majority of patients with sepsis (3) and for a high hospital mortality rate of 66%. These six subjects did not differ by center or year of enrollment.

Metabolic profiles of these 6 subjects differed markedly from the other 23 patients (13 with hydrostatic edema, 10 phenotyped as ARDS). Univariate analysis revealed that nearly one-third of the detected metabolites (250 metabolites of the 749 identified) differed in these six ARDS subjects, with $P <$

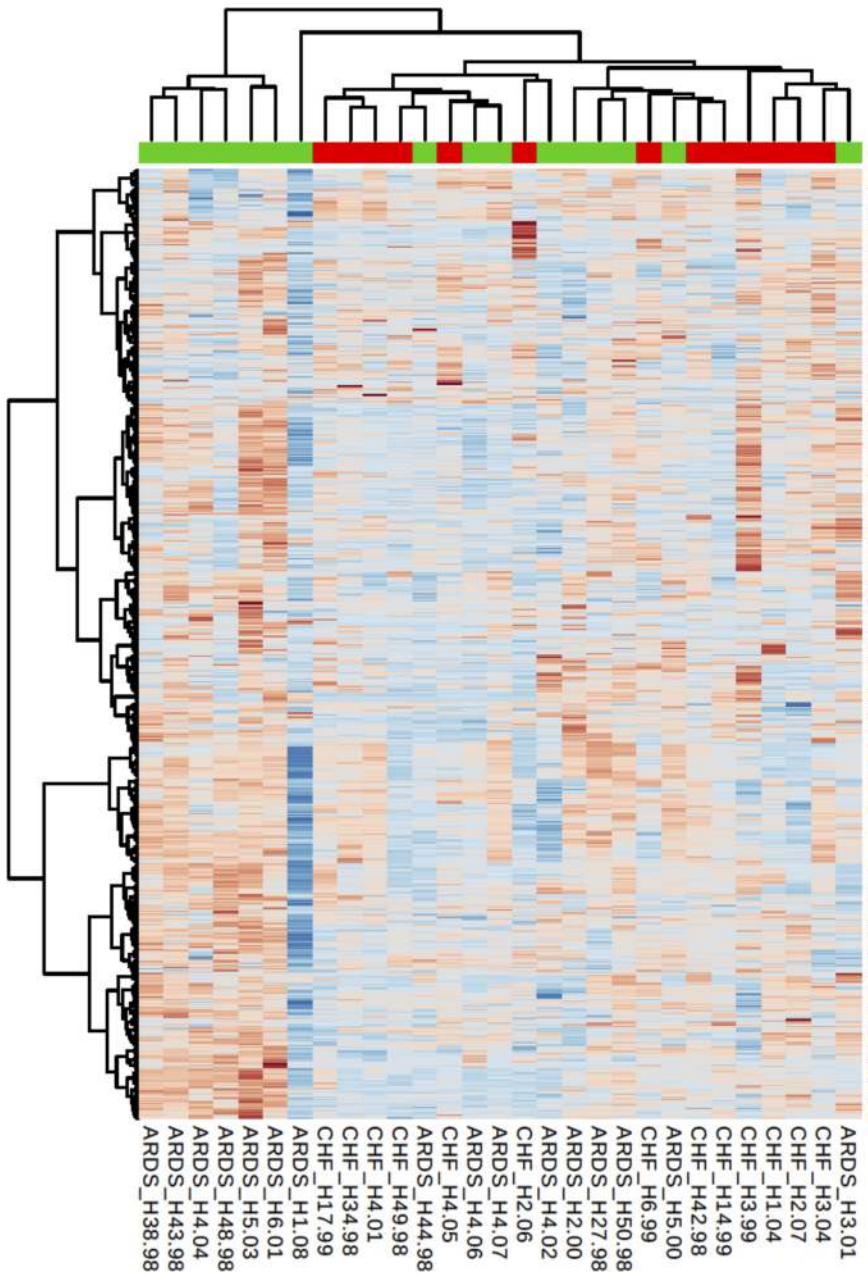

Fig. 1. Hierarchical clustering reveals widespread upregulation of metabolites in a subset of 6 acute respiratory distress syndrome (ARDS) subjects (*far left*). The other 10 ARDS edema samples (denoted in green) cluster among hydrostatic edema samples (red). CHF, chronic heart failure.

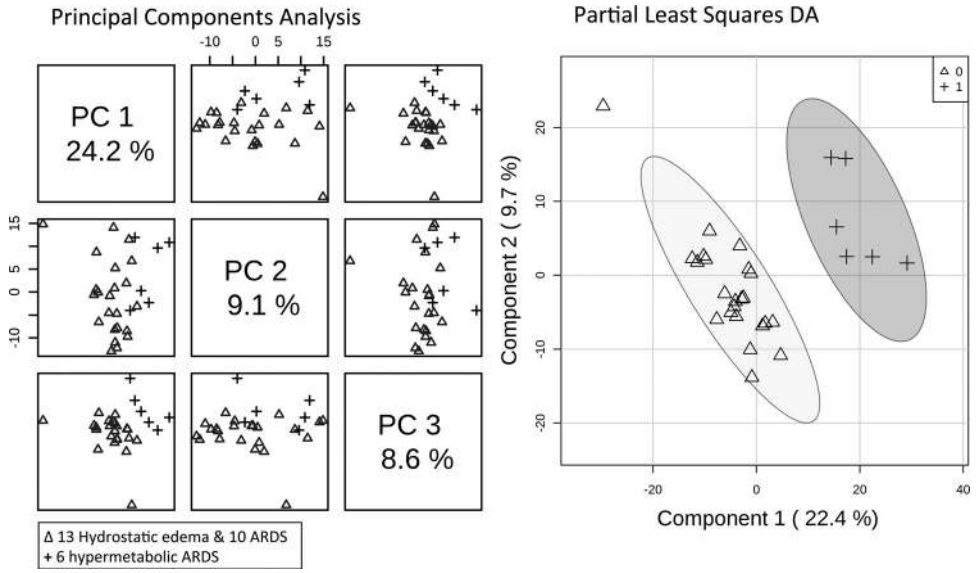

Fig. 2. Principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) results. PCA reveals partial separation of 6 hypermetabolic ARDS patients (+) vs. the 13 hydrostatic edema and other 10 ARDS patients (Δ). These groups are well separated by PLS-DA.

0.1 and fold change of at least 2 (see full list in Supplemental Table S1; Supplemental Material for this article is available online at the Journal website). The overwhelming majority of differential metabolites (235 of 250) were present at higher rather than lower levels in these six patients (Figs. 1 and 3). Differential metabolites belonged to all of the main metabolic chemical classes, including lipids, proteins, and carbohydrates (Table 3). The metabolites that contributed most to PLS-DA separation of these six patients from the others are shown in Table 4. We observed that the edema fold change for many of these metabolites was profound, with metabolite levels up to 70 times higher in edema fluid from the 6 distinct ARDS patients in comparison with the other 10 ARDS and the 13 hydrostatic edema samples.

Importantly, the widespread changes in metabolomics profiling in the six distinct ARDS patients could not be explained simply by a greater leakage of protein and metabolites from the plasma across the alveolar capillary barrier or by a more concentrated edema fluid sample. Edema fluid total protein levels were available for 15 of the 16 ARDS samples (5 of the 6 distinct ARDS samples). The edema fluid total protein levels in the 5 ARDS patients with the high metabolite profile were indistinguishable from the edema fluid total protein levels in the 10 ARDS patients without the high metabolite profile (median total protein level of 3.1 g/100 ml vs 4.0 g/100 ml, $P_{\text{Wilcoxon}} = 0.8$, Table 2). To assess whether adjustment of metabolite levels for edema fluid total protein level substantially alters the edema fluid metabolite findings, we repeated the univariate analysis comparing the 5 of 6 distinct ARDS samples for which edema fluid protein level was available with the other 23 samples. In this analysis, 207 metabolites were significantly different without adjustment for protein with $P < 0.1$, fold change >2; 153 were significantly different after adjustment for edema fluid total protein level. Of these 153 significant protein-adjusted metabolites, 136 (89%) were significant in both adjusted and unadjusted analyses, all in a consistent direction of change. Involvement of all metabolic classes was unchanged. Thus the high metabolite edema fluid profile in the distinct ARDS subset is not explained by differences in sample concentration.

Pathway analysis. The widespread metabolic differences in the six distinct ARDS edema samples involved all classes of metabolites (Table 3). To determine whether any metabolic pathway was overrepresented, we next performed a pathway analysis. Among the 250 significant metabolites, 184 had an HMDB identifier and could thus be studied for inclusion.

Alanine, aspartate, and glutamine metabolism was the only overrepresented pathway that met multiple comparisons cor-

Table 2. *Demographics of 6 ARDS patients with hypermetabolic edema fluid vs. the other 10 ARDS patients without a hypermetabolic profile*

Clinical Characteristics	Hypermetabolic Subset ($n = 6$)	Remaining ARDS ($n = 10$)	P value
Age	39 [29, 41]	49 [39, 61]	0.14
Sex, %men	66%	40%	0.61
Vasopressor	83%	70%	1.0
Mortality	66%	30%	0.30
Edema fluid:plasma protein ratio	0.9 [.76, 0.94]	0.83 [76, 0.97]	0.81
Primary diagnosis			0.12
Type (no. of cases)	Sepsis (3); fulminant hepatic failure (1); anaphylaxis (1), aspiration (1)	Pneumonia (4), sepsis (1), anaphylaxis (1), TRALI (2), reperfusion edema (1), tumor lysis (1)	

Median [IQR], with P value by Wilcoxon's rank sum for continuous variables and Fisher's exact for categorical variables, is shown. TRALI, transfusion-associated lung injury; Sepsis, nonpulmonary source of sepsis.

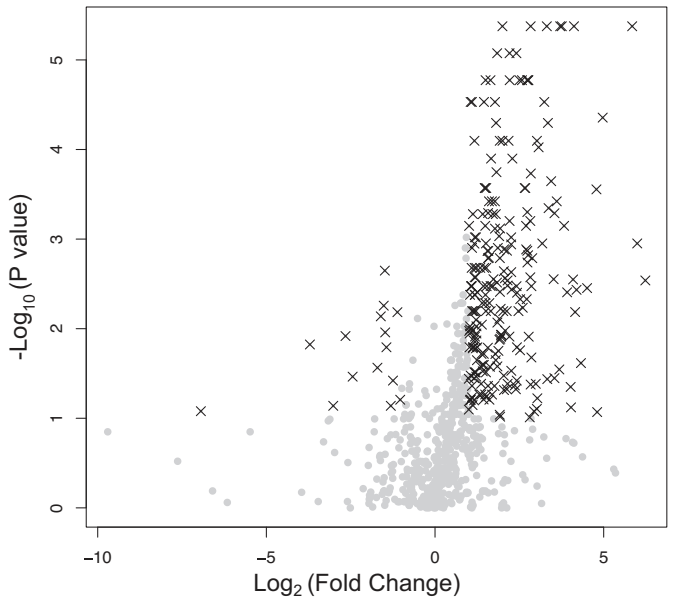

Fig. 3. Volcano plot of univariate results for the 6 metabolically active samples vs. the other 23. As shown, the vast majority of differential metabolites are upregulated in this subset (235 of 250 that meet our threshold of $P < 0.1$ and fold change > 2).

rection with FDR <0.05. As shown in Table 5, the top three pathways were all relevant to amino acid metabolism, including alanine, aspartate, and glutamate metabolism, lysine degradation, and arginine and proline metabolism pathways. Many of these pathways have been previously recognized as dysregulated in the setting of sepsis (9, 11) and whether these same pathway abnormities would have been reflected in plasma is not known.

Metabolomics of pulmonary edema fluid comparing ARDS to hydrostatic edema. We also assessed whether metabolic changes in edema fluid could be used to classify patients as having ARDS or hydrostatic edema. Not surprisingly, given the overlapping dendrogram in Fig. 1, metabolic changes in edema fluid were not sufficient to differentiate the 16 subjects with ARDS from the 13 with hydrostatic edema. No individual metabolite was significant after multiple comparisons testing (the top metabolite was sphingomyelin, with a fold change of 5.2 and $P = 0.0002$, FDR $P = 0.17$). In multivariate analysis, the PCA for the top three components similarly revealed no separation of ARDS and hydrostatic edema. The q^2 value from PLS-DA was consistently <0.3, suggesting a lack of model robustness for the separation.

DISCUSSION

In this study, we tested the metabolite profiles in edema fluid of well-phenotyped patients with ARDS in comparison with patients with hydrostatic edema. Global metabolic profiles in this study indicated that a subset of one-third of ARDS subjects have a metabolically distinct edema fluid characterized by higher levels of the vast majority of metabolites. In these six subjects, nearly one-third of all measured metabolites differed, with the vast majority (96%) increased in comparison with the edema fluid of the remaining 10 ARDS and 13 hydrostatic edema fluid samples. The altered metabolites in these six subjects included members of all classes of metabolites.

Identification of a distinct subset of one-third of ARDS subjects in this cohort mirrors closely the findings of Calfee et al. (4) in which approximately one-third of ARDS subjects were classified as belonging to a distinct, hyperinflammatory class of ARDS based on clinical variables and plasma proteomics. Because plasma was not available, we could not determine whether the six patients with a high metabolite edema fluid seen in this study would have been classified as "hyperinflammatory" based on Calfee's classification system. Importantly, the distinct class of ARDS patients with high metabolite edema fluid cannot be identified simply by assessing total edema fluid protein levels.

Pathway analysis of significantly altered metabolites in the six distinct samples identified abnormalities in amino acid pathways. Our findings are congruent with the analysis by Evans et al. (6), which identified higher levels of glutamate and proline in bronchoalveolar lavage (BAL) of patients with ARDS compared with healthy controls. Amino acid synthesis and protein breakdown are known to be widely dysregulated in sepsis (5), and several of the pathways identified have been previously noted to be abnormal in plasma of patients with sepsis (11) and even associated with sepsis mortality (9). Few have studied pulmonary-specific metabolic changes in ARDS or sepsis, although alanine metabolism of lung cells does appear to be altered in animal models of sepsis (1). Pulmonary edema metabolic changes could reflect changes in pneumocyte metabolism, a change in the immune cell profile in the alveolar milieu (15), or transit via increased endothelial permeability from plasma. Thus the implications of these findings and whether metabolic changes are reflected in plasma are unknown in this data set but merit further study.

This study has several important strengths. To our knowledge, this is the first study to profile metabolites from undiluted pulmonary edema fluid. Prior metabolomics studies have compared BAL fluid from patients with ARDS to either healthy subjects or ventilated ICU controls without edema (6). In comparison to BAL fluid, undiluted pulmonary edema fluid has the advantage of negating problems with dilution that are inherent in BAL, which is, by definition, a mixture of edema fluid and saline. Edema fluid is obtained near the time of endotracheal intubation, a critical time period when differentiating the etiology and molecular phenotype of acute lung injury could enable correct classification of patients for targeted clinical trials. Clinically phenotyped hydrostatic edema samples serve as a more clinically relevant control population than BAL of healthy individuals.

Table 3. *Metabolites that differ significantly in 6 distinct ARDS samples vs. 23 other*

Class	Significantly Altered	Total Tested
Amino acid	66	187
Carbohydrate	11	28
Cofactors and vitamins	11	31
Energy	3	10
Lipid	125	319
Nucleotide	11	36
Peptide	8	22
Xenobiotics	28	127

Results for each individual metabolite tested ($n = 749$) are available in online Supplemental Table S1.

Table 4. *Top metabolites by PLS-DA class separation for the 6 distinct ARDS samples vs. all other 23 edema samples*

Metabolite	Super Pathway	Subpathway	PLS-DA VIP Score	Wilcoxon *P*	Mean 6 Metabolic ARDS	Mean 23 Other	Fold Change
5-Dodecenoate (12:1n7)	Lipid	Medium-chain fatty acid	2.3	4×10^{-6}	12.5	0.9	13.5
3-Hydroxyoctanoate	Lipid	Fatty acid, monohydroxy	2.2	0.0002	9.8	0.9	10.9
Caprate (10:0)	Lipid	Medium-chain fatty acid	2.1	2×10^{-5}	6.0	1.1	5.7
Hexadecane-dioate	Lipid	Fatty acid, dicarboxylate	2.1	4×10^{-6}	11.3	1.1	9.9
Imidazole lactate	Amino Acid	Histidine metabolism	2.1	1.7×10^{-5}	6.3	1.0	6.6
Glycochenodeoxy-cholate	Lipid	Primary bile acid metabolism	2.1	4.2×10^{-6}	113.2	2.0	57.5
Octadecanedioate	Lipid	Fatty acid, dicarboxylate	2.1	1.7×10^{-5}	7.4	1.1	6.8
3-Hydroxylaurate	Lipid	Fatty acid, monohydroxy	2.1	4.2×10^{-6}	16.6	1.0	17.4
Docosatrienoate (22:3n3)	Lipid	Polyunsaturated fatty acid (n3 and n6)	2.1	0.0002	1.8	0.5	3.5
Ribitol	Carbohydrate	Pentose metabolism	2.1	0.0011	8.2	0.9	9.1
2-Hydroxy-3-methylvalerate	Amino acid	Leucine, isoleucine, and valine metabolism	2.0	1.7×10^{-5}	5.0	0.8	6.0
16-Hydroxypalmitate	Lipid	Fatty acid, monohydroxy	2.0	4.2×10^{-6}	4.4	1.1	4.0
Phenyllactate (PLA)	Amino acid	Phenylalanine and tyrosine metabolism	2.0	5×10^{-5}	10.4	1.0	10.2
Tauroursodeoxy-cholate	Lipid	Secondary bile acid metabolism	2.0	9.5×10^{-5}	1.7	0.2	8.4
Taurochenodeoxy-cholate	Lipid	Primary bile acid metabolism	2.0	0.0003	59.0	2.1	27.6
3-Hydroxy-decanoate	Lipid	Fatty acid, monohydroxy	2.0	4.2×10^{-6}	12.5	1.0	13.1
10-Undecenoate (11:1n1)	Lipid	Medium-chain fatty acid	2.0	1.7×10^{-5}	6.9	1.0	6.8
Myristoleate (14:1n5)	Lipid	Long-chain fatty acid	2.0	4.2×10^{-6}	6.2	0.9	7.1

PLS-DA, partial least squares discriminant analysis; VIP, variable importance in projection.

Other strengths of this study include the phenotyping of the patients by two ARDS experts (L. B. Ware and M. A. Matthay) including review of both extensive clinical data and the edema fluid-to-plasma protein ratio. Thus the phenotyping was as close to "gold standard" as is possible using clinical phenotyping.

Finally, metabolomic profiling by Metabolon has been performed in more than 1,000 publications to date, with many metabolites independently validated by outside groups, and has the potential to identify more than 3,700 metabolites. Thus this study had the potential to identify differential metabolites across a large spectrum of the known metabolites in human plasma that could have been present in edema fluid.

Our inability to separate ARDS edema fluid from hydrostatic edema fluid based on metabolic profiling is an intriguing finding. Indeed, the majority of the ARDS group ($n = 10$) had a fairly bland metabolic profile. It is certainly possible that there is more overlap between hydrostatic edema and ARDS than commonly recognized. The National Institutes of Health ARDS Network FACTT, designed to test whether liberal or conservative fluid management strategies were superior in patients with ARDS, found that 29% of ARDS patients had an initial pulmonary artery occlusion pressure of >18 mmHg, suggesting the possibility of overlap between the syndromes or even some degree of misclassification (13).

A limitation of our study is the modest sample size, although our sample size of 29 total patients is comparable to most human metabolomics studies in ARDS to date (6, 16, 17). It is possible that a much larger sample size would have provided better power to identify individual metabolites that differed between hydrostatic pulmonary edema and ARDS. No individual metabolite differed enough between all 16 ARDS subjects and the 10 with hydrostatic edema to meet correction for multiple comparisons in this study. Nonetheless, despite our modest sample size, the metabolic changes in the edema fluid of approximately one-third of patients were profound enough to drive statistically significant differences between these high metabolite ARDS patients and all others. With the exception of these six subjects who were clearly separated, the dendrogram of the cluster algorithm does not suggest a problem with power; instead, most ARDS subjects clustered with patients with hydrostatic edema due to heart failure or intravascular volume overload. Thus the possibility that phenotyping alone does not fully separate ARDS from hydrostatic edema is unlikely to result from low power. Another important limitation of this study is our inability at this time to replicate our findings in an independent population because clinical cohorts with banked samples of undiluted pulmonary edema fluid are not widely available for metabolic profiling. Thus whether the estimate that approximately one-third of ARDS patients can be characterized with a distinct metabolomic edema profile is generalizable is not certain.

In summary, in this cohort of 29 critically ill patients with acute onset of pulmonary edema, we identified a subset of approximately one-third of ARDS patients with marked metabolic differences. ARDS patients as a whole could not be separated from those with hydrostatic edema based on metabolomic profiling of edema fluid despite expert clinical phenotyping. The results of this study support the potential of metabolomics to identify distinct subsets of patients with ARDS.

Table 5. *Pathway analysis of differential metabolites that distinguish the 6 ARDS edema samples from the 23 others*

Pathway Name	Total Metabolites Tested	Significant Metabolites	*P* Value	FDR
Alanine, aspartate, and glutamate metabolism	24	7	1.4×10^{-4}	0.01
Arginine and proline metabolism	77	10	0.004	0.19
Lysine degradation	47	7	0.008	0.22

FDR, false discovery rate.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the author(s).

AUTHOR CONTRIBUTIONS

A.J.R., M.W., and L.B.W. performed experiments; A.J.R., K.C., M.W., M.Z., and M.A.M. analyzed data; A.J.R., K.C., M.W., M.Z., G.P., L.B.W., and M.A.M. interpreted results of experiments; A.J.R. prepared figures; A.J.R. drafted manuscript; A.J.R., K.C., L.B.W., and M.A.M. edited and revised manuscript; A.J.R., K.C., M.W., M.Z., G.P., L.B.W., and M.A.M. approved final version of manuscript.

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