

Untargeted LC-MS Metabolomics of Bronchoalveolar Lavage Fluid Differentiates  
Acute Respiratory Distress Syndrome from Health

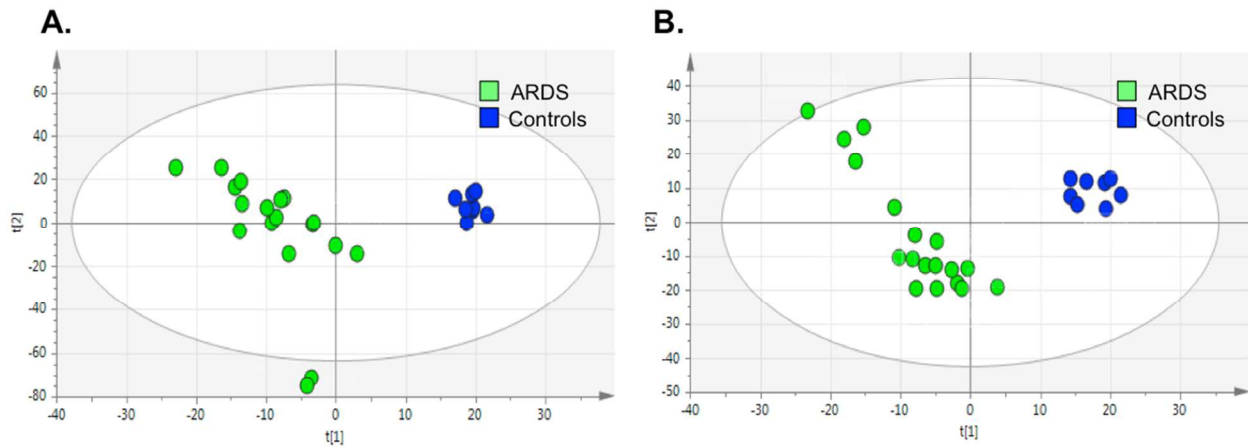
Charles R. Evans, Alla Karnovsky, Melissa A. Kovach, Theodore J. Standiford,  
Charles F. Burant, and Kathleen A. Stringer

**MATERIALS AND METHODS**

Protein Electrophoresis

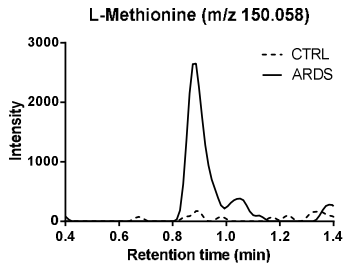
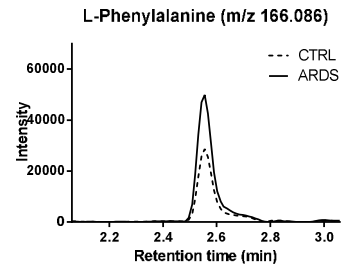
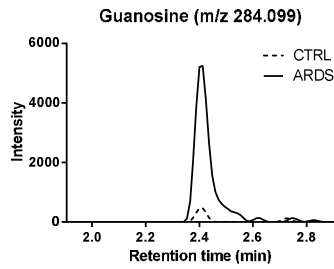
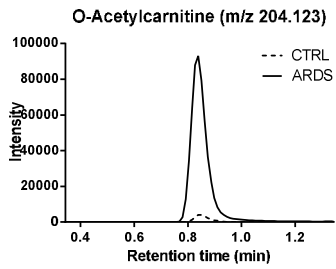
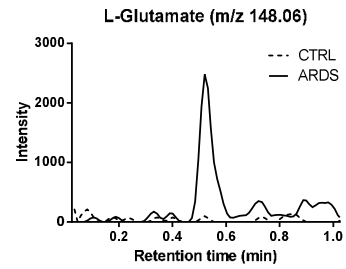
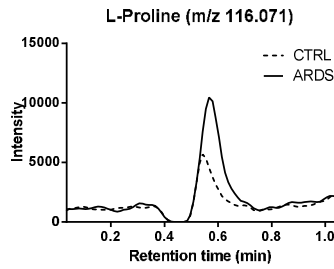
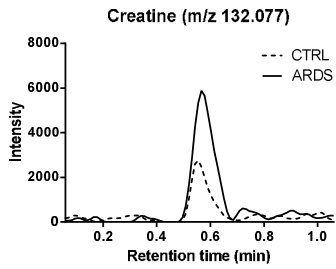
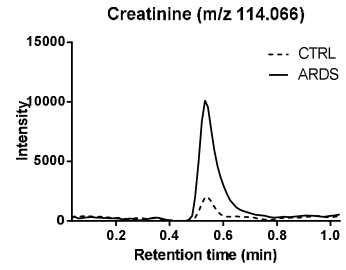
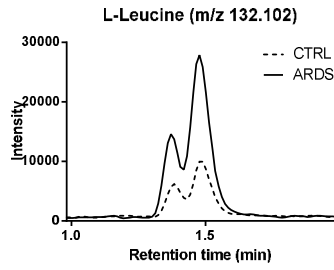
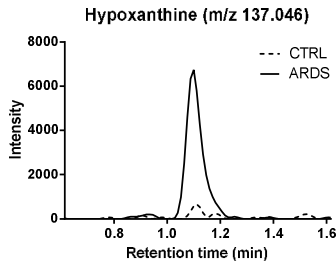
To characterize the heterogeneity of the ARDS BALF samples, protein concentration was measured using a bicinchoninic acid (BCA) assay (Pierce Protein Biology Products, Thermo Fisher Scientific, Rockford, IL USA). BALF proteins were separated by SDS-PAGE on a 12.5% acrylamide gel. Following electrophoresis, the gel was fixed in 50% ethanol (v/v) and 10% acetic acid (v/v) in ddH<sub>2</sub>O for 1 h then it was washed with gel-washing solution (50% methanol (v/v) and 10% acetic acid (v/v) in ddH<sub>2</sub>O) overnight with gentle agitation. Following aspiration of the wash buffer, the gel was submerged in Coomassie stain (0.1% Coomassie blue R350 (w/v), 20% methanol (v/v), and 10% acetic acid (v/v) in ddH<sub>2</sub>O) for 4 h with gentle agitation. The Coomassie stain was aspirated and the gel was destained in 50% methanol (v/v) and 10% acetic acid (v/v) in ddH<sub>2</sub>O. The destaining solution was exchanged several times until the background stain was minimized and the protein bands were clearly visible.

**Supplemental Figure S1:** Cross-validated PLS-DA score plots for positive (A) and negative (B) mode data ( $R^2Y = 0.99$  and  $Q^2 = 0.87$  for positive mode;  $R^2Y = 0.98$  and  $Q^2 = 0.78$  for negative mode).

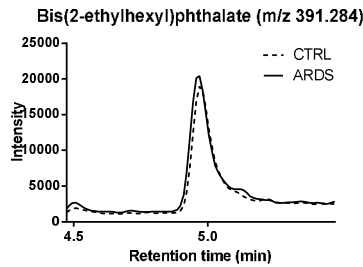
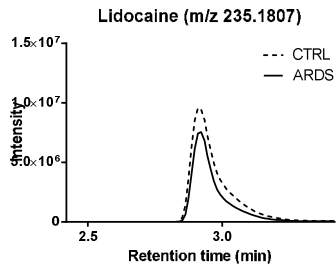


**Supplemental Figure S2:** Extracted ion chromatograms for positively identified metabolites in BALF. Chromatograms contain data from one randomly selected ARDS subject and one control subject. Due to variability in BALF metabolite levels between subjects, the peak areas do not exactly reflect the average fold-change values presented in Tables 2 and 3, and some peaks may appear with lower signal/noise than the average for the metabolite.

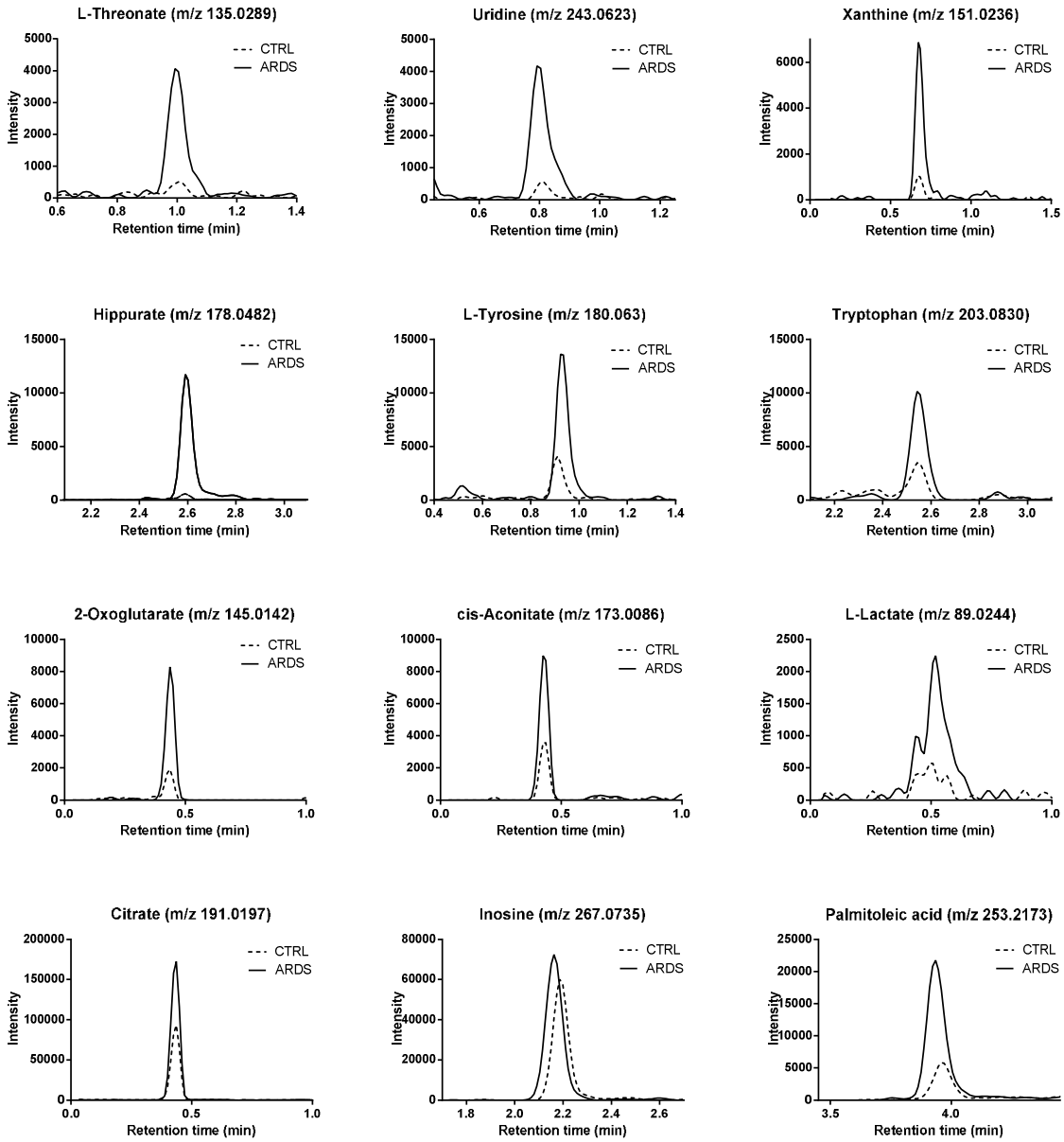
# RPLC positive ion mode extracted ion chromatograms



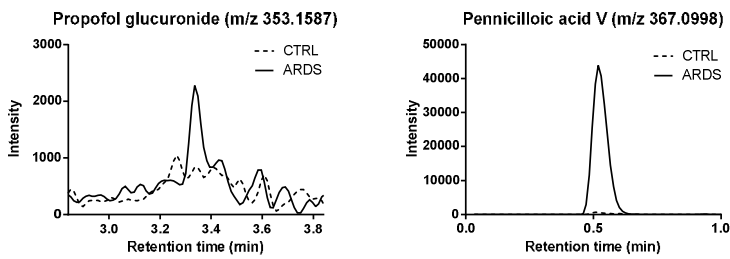
## Non-endogenous metabolites



# RPLC negative ion mode extracted ion chromatograms

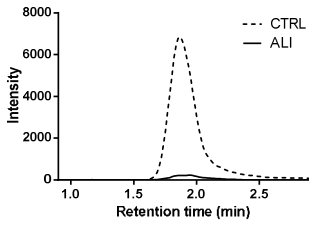


## Non-endogenous metabolites

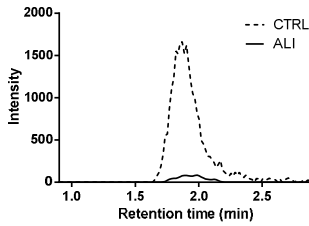


# HILIC, negative ion mode extracted ion chromatograms

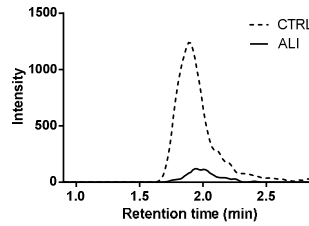
Phosphatidylcholine 16:0/16:0 (m/z 792.5637)



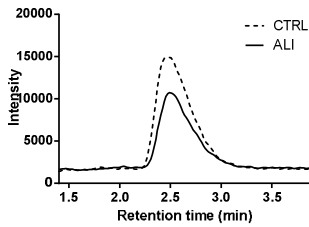
Phosphatidylcholine 16:1/16:0 (m/z 790.5568)



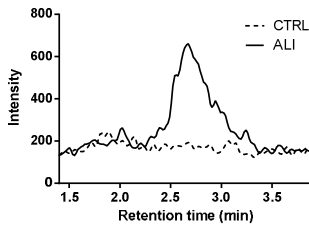
Phosphatidylcholine 16:0/14:0 (m/z 764.5434)



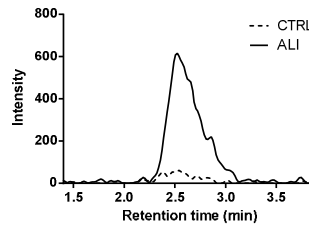
Stearic acid (m/z 283.2626)



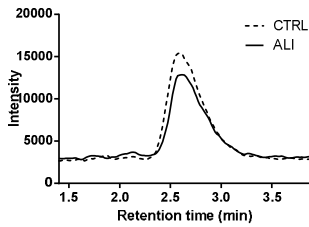
Palmitoleic acid (m/z 253.2169)



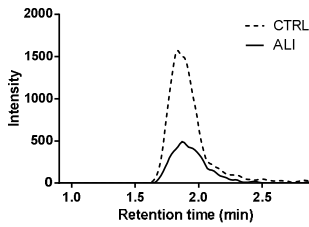
Arachidonic acid (m/z 303.2313)



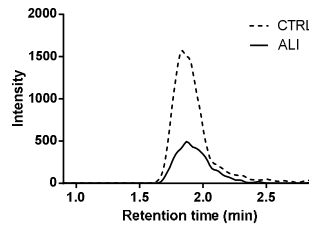
Palmitic acid (m/z 255.2313)



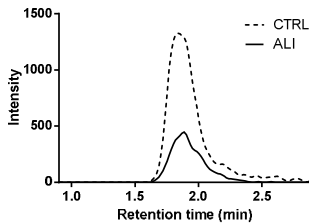
Phosphatidylcholine 16:1/18:2 (m/z 814.5595)



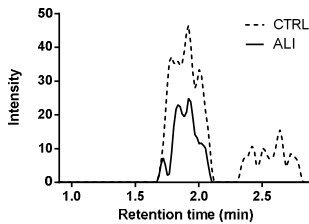
Phosphatidylcholine 16:0/18:1 (m/z 818.5753)



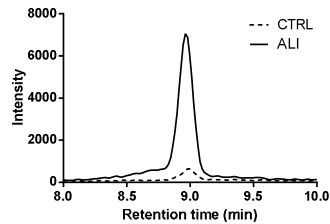
Phosphatidylcholine 16:0/18:0 (m/z 820.5693)



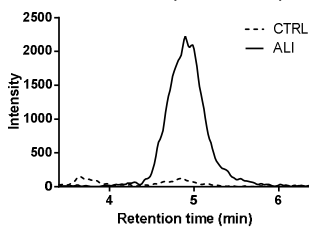
Phosphatidylcholine 16:1/20:4 (m/z 838.5511)



Lactic acid (m/z 89.024)



D-Glucose (m/z 179.0557)



Linoleic acid (m/z 279.2323)

