Adult respiratory distress syndrome (ARDS) is a non-cardiogenic pulmonary edema of various etiologies. Here we report the first application of proton nuclear magnetic resonance (NMR) for the detection of abnormal metabolites in plasma from patients with ARDS. By comparing plasma obtained from the systemic artery with that obtained from the pulmonary artery, we could study the metabolic status of the lung in patients with ARDS. Although their concentrations may vary, the peaks for acetate, acetoacetate, \( \beta \)-hydroxybutyrate, phenylalanine, and other unidentified compounds in water-suppressed NMR of these patients' plasma were higher than in the normal controls. The proton NMR resonance at a chemical shift of about 7.4 ppm (relative to sodium tetradeuterio-3-trimethylsilylproponate), presumably caused by phenylalanine and its related metabolites produced by a disordered amino acid metabolism, is detected in >65% of the samples from ARDS patients. We discuss the detection of abnormal metabolites in terms of possible deranged metabolism of carbohydrates, lipids, or amino acids in this syndrome.

**Additional Keyphrases:** lung disease · phenylalanine

Adult respiratory distress syndrome (ARDS) is a catastrophic illness with a high mortality rate; it can be provoked by many illnesses, including sepsis of various origins, aspiration pneumonia, and bone fracture (1). Multiple cellular factors, such as neutrophils, and humoral factors, such as derivatives of arachidonic acid, are believed to be involved in the pathogenesis of ARDS (2). Leaks in the pulmonary endothelium and epithelium, perhaps due to metabolic changes induced by these factors, are currently believed to be the mechanism of the pathogenesis of non-cardiogenic pulmonary edema. By comparing the composition of the arterial plasma of the patients with that of normal controls, we may be able to detect abnormal metabolites in the blood of patients with ARDS. Furthermore, by comparing the composition of the arterial plasma with that of the mixed venous plasma, we may be able to distinguish the detected metabolites from xenobiotic compounds. The mixed venous plasma obtained at the same time can serve as a control in the same patient for the detection of changes in the arterial plasma.

Nuclear magnetic resonance (NMR) is well suited for non-destructive screening of biomaterials, and involves minimal sample pretreatment (3–8). However, the determinations of concentrations of metabolites by NMR are hindered by the binding of the metabolites to plasma proteins (9), variations in linewidth, and the low sensitivity of NMR in comparison with other analytical methods (e.g., HPLC and gas chromatography). Because the metabolic status of ARDS patients may vary greatly and rapidly, a quick and easy diagnostic method is required to monitor such changes. We examined water-suppressed proton NMR as a tool for screening metabolites in plasma samples from ARDS patients.

**Materials and Methods**

Included in this study were 17 patients diagnosed as having ARDS (of known etiologies). Both an arterial line and a Swan–Ganz catheter were inserted when the patients were first seen in the Intensive Care Unit of the Veterans General Hospital, Taipei, for the measurement of systemic arterial pressure, pulmonary capillary wedge pressure, cardiac output, and so on. From both sampling sites, we collected 10 mL of blood from each patient. After thorough mixing with heparin, the blood was centrifuged at 3000 \( \times g \) for 20 min to eliminate its cellular components, and the plasma thus obtained was stored at \(-80 \degree C\) for later NMR studies and protein measurements. As a control, we also obtained arterial plasma from nine healthy volunteers by

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5. Nonstandard abbreviations: ARDS, adult respiratory distress syndrome; NMR, nuclear magnetic resonance; and TSP, tetradeutero-3-trimethylsilylproponate.

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direct puncture, and treated these samples in the same fashion for later studies.

Before NMR spectroscopy, 10% D$_2$O was added to the plasma for internal lock, and known amounts of sodium tetradeutero-3-trimethylsilylpropionate (TSP) were added to serve as internal reference peak. The plasma samples taken from the Swan–Ganz catheter and the arterial line were then processed on the Bruker AM-400 spectrometer. With the help of presaturation technique, we obtained water-suppressed proton NMR spectra after 400 scans with a 2-s delay time. A compound was assumed to be present in a sample if the signal-to-noise ratio of the peak(s) for that compound was >2. For screening purposes we made no attempts to estimate the concentrations of the metabolites in a plasma sample, because of the possible inaccuracy caused by variations in the detection of these metabolites in the plasma sample (9) and variations in the linewidths of their signals. Peak-height measurements were performed relative to creatinine or methylene when necessary. Peak identifications were assigned according to the literature (3–8), the method of standard additions, and HPLC purification.

For HPLC purification, we first separated 10 to 30 mL of plasma into two fractions by using Amicon ultrafiltration stirred cells (Model 8050; Amicon Div., W. R. Grace & Co., Danvers, MA) with a membrane (YM10 Diaflo ultrafilters, Amicon) that permits the transit of molecules with relative molecular mass less than 10 000. The filtrate was then lyophilized, redissolved in pure methanol, and filtered through glass fibers to eliminate the remaining proteins. After evaporation the filtrate was dissolved in de-ionized water and filtered through a Millipore membrane. HPLC (L-6000/L-6200 series; Hitachi Ltd., Tokyo, Japan) purification was carried out on a C18 reversed-phase column (4.6 mm × 25 cm, Inertsil 10 ODS, Vercopak) with a linear methanol gradient (0–100%) in 55 min. Collected fractions were then analyzed by NMR spectroscopy.

To avoid the possible errors in measuring peak intensity for broad signals by direct integration, we instead used the peak height relative to that of the internal reference to estimate the amount of a compound giving rise to that peak. Comparison of plasma composition between samples from the arterial line and the Swan–Ganz catheter was then carried out to indicate the metabolic status of the lung in ARDS.

The protein concentration of all plasma samples was determined by the method of Lowry et al. (10). We then correlated the ratio of protein concentrations for arterial to mixed venous plasma with the same ratio of peak height of a particular peak, seeking any possible relation between peak height and amount of protein in a sample.

**Results and Discussion**

For the 17 patients in the Intensive Care Unit who were diagnosed as having ARDS of various etiologies, 43 pairs of arterial and mixed venous plasma were analyzed. Figure 1 shows typical proton NMR spectra of normal plasma (Figure 1A) and of those plasma obtained from the arterial line and the Swan–Ganz catheter (Figures 1B and 1C) in an ARDS patient. The metabolic status of the lung could clearly be

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**Fig. 1.** Water-suppressed proton NMR spectrum of plasma from a normal subject (A) and a patient (B and C) with septic ARDS caused by liver abscess

Note that acetate, phenylalanine and related compounds, and several unidentified peaks at 2.12, 4.01, and 7.04 ppm (indicated by arrows) have higher intensities in the arterial plasma than those in the mixed venous plasma.
examined by comparing Figures 1B and 1C. In this case the 
intensities of the peaks due to some compounds—such as the 
peaks at chemical shifts 2.12, 2.4, 7.04, and 7.4 ppm—
increased after the blood had circulated through the lung. 
Thus, the lung must have produced those compounds and 
released them into the bloodstream. Table 1 shows the cases 
where the peak height of the peak at around 7.4 ppm was 
increased after the blood has circulated through the lung. 
The compound giving rise to peak at 7.4 ppm was purified 
and identified to be phenylalanine and related compounds, 
with the help of HPLC.

To rule out the possibility that parenterally administered 
nutrients and the many drugs used in critically ill patients 
will give rise to signals in the NMR spectra of plasma, it is 
important to compare the spectrum from the arterial plasma 
with that from the mixed venous plasma and normal 
plasma. An instance of such xenobiotic compound was 
glucose, whose concentration in plasma was sometimes 
significantly higher than that for a normal subject (see 
Figure 1, for example), yet from clinical records we knew 
that this was due to constant infusion of nutrients instead of 
disordered glucose metabolism. Keeping this in mind, we 
are able to compare plasma from ARDS patients and normal 
controls. Table 2 lists the NMR-detectable abnormal metabo-
lites in the plasma of ARDS patients in the present study. 
The lactate was observed with slightly higher probabilities 
in the NMR spectra of normal plasma, but its concentration 
was lower than that of the patients (Table 2 and Figure 1). 
In addition to lactate we have observed several peaks in the 
spectra of arterial and mixed venous plasma that did not 
appear in those of normal subjects. All or some of three 
ketone bodies have been observed in 11 of 17 patients 
with ARDS, although only one of them had documented diabetes 
mellitus. The ketone bodies in these non-diabetic individu-
als were probably caused by malnutrition or deranged 
carbohydrate metabolism. The phenylalanine peak at 
around 7.4 ppm was seen in more than 65% of ARDS 
patients, and it is possible that future similar studies can 
provide us with a simple means to screen ARDS patients.

Many metabolic abnormalities are known to be correlated 
with the progression of sepsis (11). For instance, there will 
be progressive increases in the glucose and its metabolites 
in the blood, including lactate and pyruvate; the branched-
chain amino acids leucine, isoleucine, and valine; and the 
aromatic amino acids phenylalanine and tyrosine. Also, as 
the concentration of triglycerides increases, that of acetoac-
tate decreases, and the ratio of β-hydroxybutyrate to aceto-
ate increases. Thus, increases in phenylalanine, lactate, 
acetoacetate, β-hydroxybutyrate, etc., in the plasma of 
ARDS patients are probably attributable to their metabolic 
derangement. For those patients whose ARDS was not 
caused by sepsis, metabolic abnormalities will also occur, 
because multiple system organ failure is a common mani-
festation of deteriorating ARDS (12). As for lactate, although 
not reported in ARDS or sepsis previously, it may also be 
due to the unbalanced metabolism of the patients, because it 
can be formed by oxidative decarboxylation of pyruvic acid, 
by oxidation of fatty acids, directly from certain ketogenic 
acids (13, 14), or converted from acetoacetate by 
muscle as a source of energy (14).

The approach of using NMR to probe the metabolic status 
of the lung deserves further discussion. In addition to the 
cases shown in Table 1, we also found that in some situa-
tions the intensities of the peaks could be decreased after 
the blood had circulated through the lung. To find out whether 
the leak of plasma protein into alveoli in ARDS could 
contribute to the variation in the intensities of the observed 
peaks, we measured plasma proteins by the method of 
Lowry et al. (10). We found no correlation between 
the concentration of plasma protein and the intensities of 
peaks (data not shown), thus ruling out protein as the cause 
of variation in the intensities of the observed peaks. Bell et al. 
(9) had shown that only about one-third of the lactate is 
visible in the spectrum, and that in the presence of disrup-
tive agents such as ammonium chloride, the intensity of 
lactate can be significantly increased, even though the 
amount of either lactate or plasma protein is unchanged. 
Thus, the changes in the observed intensities of the 
compounds such as lactate, acetoacetate, phenylalanine, etc., 
in the arterial and mixed venous plasma might be related not 
only to their tissue production rate and tissue elimination 
rate, but also to some unrecognized plasma ions and pro-
teins.

In conclusion, we demonstrated that proton NMR is 
useful in monitoring the metabolic status of the lung and 
the detection of abnormal metabolites in plasma of ARDS 
patients, provided that arterial and mixed venous plasma 
can be compared. Because ARDS patients are constantly in 
severe cardiopulmonary stress, understanding their meta-

### Table 1. Conditions Related to Adult Respiratory 
Distress Syndrome Associated with Increased Relative 
Peak Height for the Peak at 7.4 ppm in the NMR 
Spectra of Paired Plasma

| Patient | Maximal increment, %
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>120.3</td>
</tr>
<tr>
<td>2</td>
<td>55.4</td>
</tr>
<tr>
<td>3</td>
<td>212.9</td>
</tr>
<tr>
<td>4</td>
<td>116.8</td>
</tr>
<tr>
<td>5</td>
<td>162.5</td>
</tr>
<tr>
<td>6</td>
<td>95.3</td>
</tr>
<tr>
<td>7</td>
<td>65.3</td>
</tr>
<tr>
<td>8</td>
<td>22.9</td>
</tr>
<tr>
<td>9</td>
<td>&gt;300</td>
</tr>
</tbody>
</table>

*Increment = 7.4 (arterial line) - 7.4(Swan-Ganz)/7.4(Swan-Ganz).
7.4(X) = peak height of the peak at 7.4 ppm in the NMR spectrum of sample X.*

### Table 2. Abnormal Metabolites and Their Detectability 
in the NMR Spectra of Arterial Plasma of Patients with 
Adult Respiratory Distress Syndrome

<table>
<thead>
<tr>
<th>Abnormal metabolites (peaks)</th>
<th>Patient</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>72.1</td>
<td>88.9</td>
</tr>
<tr>
<td>Acetate</td>
<td>7.0</td>
<td>0</td>
</tr>
<tr>
<td>β-Hydroxybutyrate</td>
<td>23.3</td>
<td>0</td>
</tr>
<tr>
<td>Acetone</td>
<td>16.3</td>
<td>0</td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>25.6</td>
<td>0</td>
</tr>
</tbody>
</table>
| Phenytoin and related com-
  pounds                     | 67.4    | 11.1   |
| Peptide c. peak             | 7.0     | 0      |
| Peptidic peak               | 23.3    | 0      |

*For screening purposes the concentrations of these metabolites were not 
estimated because of the possible inaccuracy due to variations in the binding 
of these metabolites to the plasma proteins (see reference 9, for example) 
and the variations in their linewidths.*

*Others are assumed to be present in a sample if the signal/noise ratio of 
the peak(s) due to that compound is >2 after 400 scans in a 400-kHz 
spectrometer.*
bolic condition by using such a convenient tool as NMR spectroscopy may be important in treating these patients.

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References