Respiratory Research

Surfactant disaturated-phosphatidylcholine kinetics in acute respiratory distress syndrome by stable isotopes and a two compartment model

Paola E Cogo*†1, Gianna Maria Toffolo†2, Carlo Ori†3, Andrea Vianello†4, Marco Chierici†2, Antonina Gucciardi†1, Claudio Cobelli†2, Aldo Baritussio†5 and Virgilio P Carnielli†6,7

Address: 1Department of Pediatrics, University of Padova, Padova, Italy, 2Department of Information Engineering, University of Padova, Italy, 3Department of Pharmacology, Anaesthesia and Critical Care, University of Padova, Padova, Italy, 4Respiratory Unit, General Medical Hospital, Padova, Italy, 5Department of Medical and Surgical Sciences, University of Padova, Padova, Italy, 6Neonatal Division, Salesi Children’s Hospital, Ancona, Italy and 7Nutrition Unit, Institute of Child Health and Great Ormond Street Hospital, London, UK

Email: Paola E Cogo* - cogo@pediatria.unipd.it; Gianna Maria Toffolo - toffolo@dei.unipd.it; Carlo Ori - carloori@unipd.it; Andrea Vianello - andrea.vianello@sanita.padova.it; Marco Chierici - marco.chierici@dei.unipd.it; Antonina Gucciardi - spec2@child.pedi.unipd.it; Claudio Cobelli - cobelli@dei.unipd.it; Aldo Baritussio - aldo.baritussio@unipd.it; Virgilio P Carnielli - v.carnielli@ich.ucl.ac.uk

* Corresponding author    †Equal contributors

Abstract

Background: In patients with acute respiratory distress syndrome (ARDS), it is well known that only part of the lungs is aerated and surfactant function is impaired, but the extent of lung damage and changes in surfactant turnover remain unclear. The objective of the study was to evaluate surfactant disaturated-phosphatidylcholine turnover in patients with ARDS using stable isotopes.

Methods: We studied 12 patients with ARDS and 7 subjects with normal lungs. After the tracheal instillation of a trace dose of 13C-dipalmitoyl-phosphatidylcholine, we measured the 13C enrichment over time of palmitate residues of disaturated-phosphatidylcholine isolated from tracheal aspirates. Data were interpreted using a model with two compartments, alveoli and lung tissue, and kinetic parameters were derived assuming that, in controls, alveolar macrophages may degrade between 5 and 50% of disaturated-phosphatidylcholine, the rest being lost from tissue. In ARDS we assumed that 5–100% of disaturated-phosphatidylcholine is degraded in the alveolar space, due to release of hydrolytic enzymes. Some of the kinetic parameters were uniquely determined, while others were identified as lower and upper bounds.

Results: In ARDS, the alveolar pool of disaturated-phosphatidylcholine was significantly lower than in controls (0.16 ± 0.04 vs. 1.31 ± 0.40 mg/kg, p < 0.05). Fluxes between tissue and alveoli and de novo synthesis of disaturated-phosphatidylcholine were also significantly lower, while mean resident time in lung tissue was significantly higher in ARDS than in controls. Recycling was 16.2 ± 3.5 in ARDS and 31.9 ± 7.3 in controls (p = 0.08).

Conclusion: In ARDS the alveolar pool of surfactant is reduced and disaturated-phosphatidylcholine turnover is altered.
Background
ARDS is a syndrome of reduced gas exchange due to a diffuse injury to the alveolar capillary barrier and is characterized by filling of the alveoli with proteinaceous fluid, infiltration by inflammatory cells and consolidation [1]. It may develop after a direct insult to the lung parenchyma or it may result from inflammatory processes carried into the lungs via the pulmonary vasculature. In the early exudative phase of ARDS the massive, self-perpetuating inflammatory process is characterized by an increased endothelial and epithelial permeability with leakage of plasma components.

Constriction and microembolism of the pulmonary vessels are also present, leading to ventilation perfusion mismatch. Moreover an increase in the alveolar surface tension causes alveolar instability, atelectasis and ventilatory inhomogeneities. In severe ARDS, just a small fraction of parenchyma remains aerated, and the damage can be so widespread that normal parenchyma, as judged by computed tomography, may shrink to 200–500 g [2,3].

One of the hallmarks of ARDS is reduced lung compliance and loss of stability of terminal airways at low volumes, suggesting surfactant dysfunction or deficiency. Samples of bronchoalveolar lavage fluid from patients with ARDS have low concentrations of disaturated-phosphatidylcholine, phosphatidylglycerol and surfactant-specific proteins and fail to reduce surface tension both in vitro and in vivo [4,5]. Surfactant organization in the alveoli is also altered, since large aggregates, the active fraction of surfactant, decrease in patients with ARDS [6]. To our knowledge, the alveolar pool of surfactant has never been rigorously estimated in patients with ARDS, nor is it known if surfactant turnover is altered in this condition.

Data on surfactant metabolism in ARDS are available from animal studies which showed a faster turnover rate and a decreased alveolar pool of disaturated-phosphatidylcholine, while the tissue pool was increased in some studies and unchanged in others [7-9]. However these experiments cannot be repeated in humans and may not necessarily mimic human disease.

In this paper we studied the turnover of surfactant disaturated-phosphatidylcholine in patients with ARDS and in control subjects. To this end we instilled a trace dose of $^{13}$C-dipalmitoyl-phosphatidylcholine into the trachea and then followed over time the $^{13}$C enrichments in disaturated-phosphatidylcholine-palmitate isolated from serial tracheal aspirates.

Available evidence indicates that surfactant dipalmitoylphosphatidylcholine is recycled several times before being degraded by alveolar macrophages or within lung parenchyma [7]. There is uncertainty, however, about the contribution of alveolar macrophages to surfactant catabolism, since animal experiments indicate that alveolar macrophages could degrade between 5 and 50% of surfactant disaturated-phosphatidylcholine [10,11]. In patients with ARDS, the fraction of disaturated-phosphatidylcholine degraded in the alveolar space could be even greater than this, due to the presence of inflammatory cells, bacteria and free hydrolytic enzymes [12,13]. On the basis of these considerations we assumed that alveolar macrophages may degrade 5–50% of saturated phosphatidylcholine in controls and 5–100% in patients with ARDS.

Methods
Patients
We studied 12 adult patients with ARDS, defined according to Bernard [14], and 7 subjects with normal lungs on mechanical ventilation or breathing spontaneously through a tracheostomy tube due to neuromuscular diseases. Patients were admitted to the Intensive Care or Respiratory Units of the University of Padova, Italy. The study was approved by the Ethics Committee, and written, informed consent was obtained. After intubation with a cuffed tube, all patients received into the trachea 20 ml of normal saline containing 7.5 mg of $^{13}$C-dipalmitoyl-phosphatidylcholine and 40 mg of surfactant extract (Curosurf®, Chiesi, Parma, Italy) as spreading agent. Both palmitates were uniformly labeled with carbon 13 ([U-$^{13}$C-PA]-DPPC, Martek-Biosciences, Columbia, MD). The suspension was instilled close to the carina with a 4.5 mm bronchoscope (Olympus BF-40 OD 6.0 mm Olympus-Europe, Italy). Patients with ARDS were studied within 72 h from the onset of the acute respiratory failure and ventilator parameters were adjusted to maintain an oxygen saturation $>$ 85% and pH $>$ 7.25. Ventilator and gas exchange parameters were recorded at time 0 and subsequently every 6 h in ARDS patients and at least once in controls.

Study design
Tracheal aspirates, collected by suction below the tip of the endotracheal tube after instilling 5 ml of normal saline, were obtained at baseline, every 6 h until 72 h and then every 12 h for 7 days or until extubation. Aspirates were filtered on gauze, centrifuged at 150-g for 10 minutes and supernatants were stored at -20°C.

Analytical methods
Lipids from tracheal aspirates and from the administered tracer were extracted according to Bligh and Dyer after addition of the internal standard heptadecanoylphosphatidylcholine [15]. One third of the extract was oxidized with osmium tetroxide. Disaturated-phosphatidylcholine was isolated from the lipid extract by thin layer chromatography [16], the fatty acids were derivatized with osmium tetroxide. Disaturated-phosphatidylcholine-palmitate isolated from serial tracheal aspirates were extracted according to Bligh and Dyer after addition of the internal standard heptadecanoylphosphatidylcholine [15]. One third of the extract was oxidized with osmium tetroxide. Disaturated-phosphatidylcholine was isolated from the lipid extract by thin layer chromatography [16], the fatty acids were derivatized with osmium tetroxide. Disaturated-phosphatidylcholine-palmitate isolated from serial tracheal aspirates were extracted according to Bligh and Dyer after addition of the internal standard heptadecanoylphosphatidylcholine [15]. One third of the extract was oxidized with osmium tetroxide. Disaturated-phosphatidylcholine was isolated from the lipid extract by thin layer chromatography [16], the fatty acids were derivatized with osmium tetroxide. Disaturated-phosphatidylcholine-palmitate isolated from serial tracheal aspirates were extracted according to Bligh and Dyer after addition of the internal standard heptadecanoylphosphatidylcholine [15]. One third of the extract was oxidized with osmium tetroxide. Disaturated-phosphatidylcholine was isolated from the lipid extract by thin layer chromatography [16], the fatty acids were derivatized with osmium tetroxide. Disaturated-phosphatidylcholine-palmitate isolated from serial tracheal aspirates were extracted according to Bligh and Dyer after addition of the internal standard heptadecanoylphosphatidylcholine [15]. One third of the extract was oxidized with osmium tetroxide. Disaturated-phosphatidylcholine was isolated from the lipid extract by thin layer chromatography [16], the fatty acids were derivatized with osmium tetroxide.
Diazomethylation of phospholipids was performed as described previously [6]. Phospholipid methyl esters were extracted with hexane and stored at -20°C. Phospholipid methyl esters were derivatized as pentafluorobenzyl derivatives [17], extracted with hexane and stored at -20°C. Tracheal aspirates with visible blood were discarded. The enrichments of 13C-disaturated-phosphatidylcholine-palmitate were measured by gas chromatography-mass spectrometry (GC-MS, Voyager, Thermoquest, Rodano, Milano, Italy), as previously described [18].

**Data analysis**

Data were analyzed with the two compartment model shown in figure 1 under the following assumptions: a) surfactant is distributed between two compartments (alveoli and lung parenchyma); b) disaturated-phosphatidylcholine is synthesized by lung parenchyma, secreted in the alveoli and recycled before being degraded by alveolar macrophages or lung tissue; c) the system is at steady state and is not perturbed by the administration of tracer. These assumptions have been validated in adult and newborn animals by several authors, and have been used in numerous studies on surfactant turnover in experimental animals [7,19-21].

Tracer model equations are:

\[
\begin{align*}
\bar{m}_1 (t) &= - (k_{01} + k_{21}) m_1 (t) + k_{12} m_2 (t) + u(t) \\
\bar{m}_2 (t) &= k_{21} m_1 (t) - (k_{01} + k_{12}) m_2 (t)
\end{align*}
\]

**Figure 1**

A two compartment model. Two compartment model for the analysis of disaturated-phosphatidylcholine-palmitate kinetics. Compartment 1 is the alveolar space, compartment 2 is lung tissue. \( M_1 \) and \( M_2 \) are tracer disaturated-phosphatidylcholine-palmitate masses, \( P \) is disaturated-phosphatidylcholine-palmitate de novo synthesis, \( F_{21} \) and \( F_{12} \) are inter-conversion fluxes, \( F_{01} \) and \( F_{02} \) are irreversible loss fluxes, \( k_{21} \) and \( k_{12} \) are interconversion rate parameters, \( k_{01} \) and \( k_{02} \) are irreversible loss rate parameters, \( u \) is the tracer disaturated-phosphatidylcholine-palmitate input in compartment 1 and the dashed line with a bullet indicates the tracer to tracee ratio (ttr) measurement. It is assumed that loss from the alveolar space is 5–50% in controls and 5–100% in ARDS.
where \( m_1 \) and \( m_2 \) are the amount (in mg) of disaturated-phosphatidylcholine-palmitate tracer in compartment 1 and 2 respectively, \( \dot{m}_1 \) and \( \dot{m}_2 \) (mg/h) represent their rate of change, \( k_{12} \) and \( k_{21} \) (h\(^{-1}\)) are inter-conversion rate parameters, \( k_{01} \) and \( k_{02} \) (h\(^{-1}\)) are irreversible losses, and \( u \) is the labeled disaturated-phosphatidylcholine-palmitate injection into the accessible compartment.

Tracee steady state equations are:

\[
0 = -(K_{01} + K_{21})M_1 + K_{12}M_2 = -F_{01} - F_{21} + F_{12}
\]

\[
0 = K_{21}M_1 - (K_{01} + K_{12})M_2 + P = F_{21} - F_{01} - F_{12} + P \quad (2)
\]

where \( M_1 \) and \( M_2 \) (mg) are the steady state tracee disaturated-phosphatidylcholine-palmitate masses in the two compartments, \( P \) (mg/h) is disaturated-phosphatidylcholine-palmitate de novo synthesis, \( F_{21} = k_{21}M_1 \), \( F_{12} = k_{12}M_2 \), \( F_{01} = k_{01}M_1 \), \( F_{02} = k_{02}M_2 \) (mg/h) are inter-conversion and irreversible loss fluxes.

Measured tracer to tracee ratio at time \( t \) is the ratio between tracer and tracee masses in the accessible compartment:

\[
tr(t) = \frac{m_1(t)}{M_1} \quad (3)
\]

The tracer model (equations 1 and 3) is not identifiable, since it is not possible to quantify from the input-output tracer experiment in the alveolar compartment unique values for the unknown parameters of the tracer model, namely \( M_1, k_{01}, k_{02}, k_{12}, k_{21} \) [22]. Only the mass in the alveolar compartment \( M_1 \) can be uniquely identified, together with some combinations of the original parameters, namely \( k_{01}+ k_{12}, k_{02} + k_{21} \) and \( k_{21} k_{12} \). To resolve model nonidentifiability, assumptions on the relative role of the two degradation pathways need to be incorporated into the model. Based on the results of studies in which rabbits or mice received non-degradable analogues of disaturated-phosphatidylcholine into the trachea [10,11], we assumed that, in normal subjects, alveolar macrophages may degrade between 5 and 50% of surfactant disaturated-phosphatidylcholine, the remaining being degraded by lung parenchyma (i.e. \( F_{01} \) varies between 5 and 50% of \( F_{01} + F_{02} \)). In ARDS, we assumed that the degradation of disaturated-phosphatidylcholine in the airways could vary between 5 and 100% due to the degradative activity of inflammatory cells, bacteria or enzymes released in the alveolar spaces (i.e. \( F_{01} \) varies between 5 and 100% of \( F_{01} + F_{02} \)). Using this information, upper and lower bounds for parameters \( k_{12}, k_{21}, k_{01} \) and \( k_{02} \) were estimated from tracer to tracee data in each individual [23]. Using these values in equation 2, upper and lower bounds were derived for \( P, M_2 \) and tracee fluxes \( F_{21} \) and \( F_{02} \), while flux \( F_{12} \) was uniquely solved [22]. Additional kinetic parameters were used to characterize the system, namely the total mass in the system (\( M_{tot} = M_1 + M_2 \)), the mean residence time of molecules entering the system from alveoli or lung tissue (\( MRT_1, MRT_2 \)), defined as the sum of the elements in column 1 and 2 of the mean residence time matrix \( \Theta \):

\[
\Theta = \begin{bmatrix} k_{01} + k_{21} & k_{12} \\ k_{21} & k_{02} + k_{12} \end{bmatrix}^{-1} \begin{bmatrix} k_{01} & k_{02} + k_{12} \\ k_{21} & k_{01} + k_{12} \end{bmatrix} \quad (4)
\]

and the percentage \( R \) (%) of particles that recycle back after leaving the intracellular pool:

\[
R = \frac{k_{21}}{k_{21} + k_{01}} \cdot \frac{k_{12}}{k_{12} + k_{02}} \quad (5)
\]

Upper and lower bound were calculated for \( M_{tot}, MRT_1, \) and \( MRT_2 \) [22], while unique values were calculated for \( R \).

Table 1: Clinical characteristics of patients with ARDS and control subjects

|                     | ARDS N = 12 | CONTROLS N = 7 | p    |
|---------------------|-------------|----------------|------|
| Body Weight (kg)    | 74 ± 16     | 58 ± 12        | 0.05 |
| Age (years)         | 60 ± 16     | 50 ± 23        | 0.37 |
| Mechanical Ventilation (days) | 23 ± 16 | 81 ± 129 | 0.21 |
| Mechanical Ventilation at the start of the study (days) | 2.6 ± 2 | 69 ± 132 | 0.23 |
| Male/Female (number) | 8/4        | 3/4            | 0.324|
| Survival (alive/total number) | 4/12     | 7/7            | 0.006|
| Mean FiO2 (percentage) | 60 ± 16   | 24 ± 14        | <0.001|
| Mean PEEP (cm H2O)  | 7.7 ± 1.8   | 1.3 ± 0.2      | <0.001|
| Mean AsDO₂ ‡        | 283 ± 129   | 52 ± 38        | <0.001|
| Mean PaO₂/FiO₂ §    | 162 ± 50    | 382 ± 79       | <0.001|

‡ AsDO₂ = Mean Alveolar-arterial oxygen gradient during the study
* PaO₂/FiO₂ = PaO₂/FiO₂ ratio during the study period
Data is presented as mean ± SD
Model identifiability
Parameters $k_{21}$, $k_{12}$, $k_{01}$, $k_{02}$, and $M_1$ of the model (figure 1) were fitted on disaturated-phosphatidylcholine-palmitate tracer to tracee ratio using SAAMII [24]. Weights were chosen optimally, i.e. equal to the inverse of the measurement errors. They were assumed to be Gaussian, independent and zero mean with a constant coefficient of variation, which was estimated a posteriori.

Masses of palmitate residues were multiplied by 1.3025 to obtain disaturated-phosphatidylcholine masses. Rate of changes ($k$), fluxes ($F$) and synthesis ($P$) were multiplied by 24 to obtain the respective values per day.

Statistical analysis
Results are presented as mean ± SEM. Data in Table 1 are presented as mean ± SD. Differences were analysed using the Mann-Whitney test with a 2-tailed probability of <0.05 (SPSS 10.0, Windows 2000). Parameters, resolved as upper and lower bounds, were considered different when the interval of admissible values in ARDS was significantly different from the interval of admissible values in controls.

Results

Clinical characteristics
We studied 12 ARDS patients and 7 controls. No ARDS patient was treated with exogenous surfactant. Eight ARDS patients (67%) died before hospital discharge, 5 for multi-organ failure and 3 for the underlying disease. Patients died within 4 to 18 days of study completion and during the study respiratory and gas exchange parameters were stable. No death occurred in the control group. In the control group, five patients suffered from spinal muscular atrophy, two had polineuropathy and one had encephalopathy secondary to head injury. Clinical characteristics of the 12 ARDS and 7 controls are reported in Table 1. ARDS was induced by an indirect insult in all but one patient (patient 5, Table 2). Mean age was comparable in the two groups, mean weight was significantly lower in control groups ($p = 0.05$) and the male/female ratio was 8/4 in ARDS and 3/4 in controls ($p = 0.26$). Ventilator parameters were significantly different as expected from the study design. All ARDS patients were mechanically ventilated, whereas six controls were on intermittent ventilator support and one was breathing spontaneously via tracheostomy tube. Table 2 reports detailed clinical data for the 12 ARDS patients.

Kinetic calculations
The average time courses of disaturated-phosphatidylcholine-palmitate tracer to tracee ratio in controls and ARDS are shown in figure 2. Although similar tracer doses were used in ARDS and controls, the tracer to tracee ratio of ARDS was markedly higher than in controls. In both cases, the tracer to tracee ratio declined to negligible values at 96 h. Therefore we used data up to 96 h.

Individual curves of the tracer to tracee ratio were fitted to the model presented in figure 1. All parameters were estimated with acceptable precision, on average less than 50%. Kinetic parameters are summarized in figure 3 and depicted in greater detail in figure 4. Three of them ($M_1$, $F_1$, and $R$) were uniquely identified, the others are presented as ranges of values included between two extremes, the upper and lower bounds.

In controls, the alveolar pool of disaturated-phosphatidylcholine was $1.31 ± 0.40$ mg/kg, far smaller than the tissue pool, which, depending on assumptions about degradation of disaturated-phosphatidylcholine by alveolar macrophages, ranged from $9.64 ± 2.43$ to $19.35 ± 3.74$ mg/kg. De novo synthesis ($P$) of disaturated-phosphatidylcholine ranged from $4.25 ± 0.7$ to $8.64 ± 1.44$ mg/kg/day.
Tracer to tracee ratio plot. Tracer to tracee ratio (ttr) in disaturated-phosphatidylcholine and palmitate isolated from tracheal aspirates in ARDS (upper) and controls (lower). Values are mean ± SEM. n = 7 for control subjects and 12 for patients with ARDS.
The flux from alveoli to tissue (F_{21}) ranged from 3.12 ± 1.49 to 4.80 ± 1.78 mg/kg/day. The flux from tissue to alveoli (F_{12}) was 5.23 ± 1.78 mg/kg/day and recycling (R) was 31.9 ± 7.3%. According to the model, labelled disaturated-phosphatidylcholine is expected to accumulate into the lung parenchyma of control subjects, reaching a maximum concentration between 12 and 24 hours after instillation. Afterwards, tissue isotopic enrichment is expected to decrease, so that 96 hours after the start of the study around 20% of the tracer remains associated with lung tissue (data not shown).

In patients with ARDS, the alveolar pool of disaturated-phosphatidylcholine (M_{1}) was smaller than in controls: 0.16 ± 0.04 vs 1.31 ± 0.40 mg/kg (p < 0.05). Fluxes between tissue and alveoli (F_{12} and F_{21}) and de novo synthesis (P) of disaturated-phosphatidylcholine were also smaller than in controls. Fractional rates of transfer between tissue and airways (k_{21} and k_{12}) and alveolar mean resident time (MRT_{1}) were not different from controls. In ARDS, the tissue mean resident time of disaturated-phosphatidylcholine was significantly longer than in controls (figure 3 and 4). Recycling tended to be smaller in patients with ARDS, but the difference was not significant: 16.2% ± 3.5 in ARDS and 31.9% ± 7.3 in controls (p = 0.08, figure 4). Differences between ARDS and control patients appear to be robust, since, with the exception of the synthesis rate P, all differences remained significant even assuming in controls 5–100% of disaturated-phosphatidylcholine can be degraded in the alveolar spaces.

The model predicts that in ARDS instilled disaturated-phosphatidylcholine associates rapidly with lung tissue, reaching a maximum after 12–24 hours, and then decreases gradually, so that after 96 hours 10–30% of the dose remains tissue-associated (not shown).

**Discussion**

Pulmonary surfactant is essential for normal lung function, and it is well established that surfactant impairment contributes to respiratory failure in ARDS [4,5,25-27]. These observations prompted the use of exogenous surfactant in ARDS, to replenish a deficient state and reverse surfactant inactivation [28-30]. However, large randomized clinical trials have given puzzling results [28-30] suggesting that other processes, besides surfactant dysfunction, may contribute to lung damage in ARDS or at least indicating that exogenous surfactant is either rapidly inactivated or is preferentially distributed to normal lung sections.

Most of our knowledge on surfactant kinetics in acute lung injury derives from animal studies done with radio-
Detailed kinetic results. Estimated and derived kinetic parameters of ARDS patients (black boxes) and controls (white boxes). Values are expressed as mean ± SEM. Symbols as in figure [1]. Stars (*) represent unique values in ARDS that were significantly lower (p < 0.05) than the respective values in controls. Crosses (†) indicate intervals of admissible values in ARDS significantly lower than in controls (upper bound in ARDS significantly lower than lower bound in controls). Double crosses (‡) indicate intervals of admissible values in ARDS significantly higher than in controls (lower bound in ARDS significantly higher than upper bound in controls).
active isotopes [7]. In this study we analysed the turnover of surfactant disaturated-phosphatidylcholine in control subjects and in patients with ARDS using stable isotopes. The technique used has been validated in pre-term baboons with bronchopulmonary dysplasia. In that experiment we found that the estimate of the alveolar and tissue pools of disaturated phosphatidylcholine obtained from the dilution of stable isotopes in tracheal aspirates compared well with direct measurements done at autopsy. [31]. The technique has been also applied to human infants with neonatal respiratory distress syndrome due to prematurity, lung malformations and infections [18,32-36]. However there are aspects of the present work, both conceptual and technical, that warrant special comment.

**Basic assumptions**

The design of the study assumes that the tracer was administered as a pulse, that there was good mixing between tracer and endogenous surfactant, that the administered material did not perturb endogenous surfactant, that tracheal aspirates were representative of events happening in the most peripheral airways and that patients were at steady state.

While in neonatal respiratory disorders the lung parenchyma is relatively homogeneous, this is certainly not the case in patients with ARDS, where areas of atelectasis and over-distension coexist and the tracer might distribute preferentially to aerated sections of the lungs [3]. In this study, to optimize distribution, we mixed the tracer with a surfactant extract used as a spreading agent. We could not document directly in our patients that the instilled material distributed uniformly throughout the aerated airways, but we relied on the following findings all indicating that the instilled material mixed well with resident surfactant: a) animals who receive surfactant through the airways with the technique we used, display a rather homogeneous distribution through the airways, [37-39]; b) our estimate of the alveolar pool of disaturated-phosphatidylcholine in control patients agrees very nicely with data obtained by Rebello et al on bronchoalveolar lavage fluid of human cadaver lungs [40]; c) in preterm baboons we found that the disaturated-phosphatidylcholine pools calculated from the dilution of tracers administered through the trachea compare well with direct measurements done at autopsy [31]; d) in the same experiment we found the disaturated-phosphatidylcholine tracer enrichments in tracheal aspirates were remarkably similar to the enrichments measured in the bronchoalveolar lavage fluid (data not shown).

The dose of disaturated-phosphatidylcholine administered to control subjects (20 ± 2 mg) represented 1.1–2.1% of the estimated lung pool [5], an amount unlikely to perturb endogenous surfactant. In patients with ARDS, the dose (20 ± 2 mg) represented 5.0–13.1% of the estimated lung pool, an amount also unlikely to induce a pharmacologic effect, considering that the doses of surfactant used for the treatment of ARDS are at least two orders of magnitude greater [29,30]. Since the dose of surfactant administered was small and clinical conditions remained stable during the study, we assume that the system was at steady state, thus allowing the use of a linear time invariant compartmental model to describe disaturated-phosphatidylcholine kinetics.

Data were analysed according to the two compartment model reported in figure 1. This model is physiologically plausible, but too complex to be uniquely resolvable from the available data, since only the mass in the alveolar compartment (M1), the flux from the lung tissue back to the alveolar space (F12) and recycling (R) can be uniquely solved. Only a far more complex experiment, with tracer administered also in the lung tissue compartment, could permit to uniquely identify all kinetic parameters. Since this experiment could not be done, we used existing knowledge on the contribution of alveolar macrophages to surfactant degradation to derive bounds for parameters that could not be uniquely identified. Thus, on the basis of animal experiments done by Gurel and Rider [10,11], we assumed that alveolar macrophages could normally degrade between 5 and 50% of surfactant disaturated-phosphatidylcholine, the remaining being degraded by the lung parenchyma. It should be noted however, that 50% degradation by alveolar macrophages probably represents a maximum, since this figure was derived on the assumption that alveolar macrophages do not re-enter the lung parenchyma after the uptake of surfactant in the alveoli [10]. In ARDS, we assumed that 5–100% of surfactant disaturated-phosphatidylcholine could be degraded in the airways, due to the degradative activity of inflammatory cells or bacteria. By incorporating these assumptions into the tracer-tracee model, upper and lower bounds were derived for all non identifiable kinetic parameters, following a strategy formalized in [23] and applied to study thyroid hormones [41,42] and glucose [43] kinetics.

**Surfactant kinetic parameters in controls**

Our estimate of the alveolar and tissue pools of disaturated-phosphatidylcholine in controls agree quite well with measurements taken by Rebello et al. during autopsies of adults without lung disease [40]. In fact, according to Rebello et al. the alveolar and tissue pools contain respectively 1.9 μmol/kg and 28.4 μmol/kg of disaturated-phosphatidylcholine. We found that in controls the alveolar pool of disaturated-phosphatidylcholine was 2.3 μmol/kg, while the tissue pool ranged between 17.1 and 34.3 μmol/kg. It is also of note that our results compare favorably with those of Martini et al. who studied sur-
factant turnover in adult pigs using stable isotopes [44]. These authors reported that mean phosphatidylcholine synthesis was 4.7 mg/kg/day, while our estimate ranged between 4.3 and 8.6 mg/kg/day. Furthermore they reported that the phosphatidylcholine tissue pool was 10 times higher than the alveolar pool [44], in good agreement with our finding that in control subjects the tissue pool was 7.6–14.8 times greater than the alveolar pool. Overall, these results support our approach and also indicate that tracheal aspirates can be as useful as bronchoalveolar lavage fluid for the study of surfactant turnover.

Using morphometric methods Young et al. estimated that the alveolar pool of disaturated-phosphatidylcholine is comparable to the lamellar body pool [45]. Thus it is likely that the tissue pool of disaturated-phosphatidylcholine measured with the present technique includes both intracellular surfactant and non-surfactant membranes that, with time, incorporate a fraction of administered disaturated-phosphatidylcholine.

**Surfactant in ARDS**

In patients with ARDS alveolar pool, fluxes between tissue and alveoli and *de novo* synthesis of disaturated-phosphatidylcholine were all smaller than in controls, while the mean residence time in lung tissue was greater than in controls. These differences appear to be robust, since, with the exception of *de novo* synthesis, they persist even assuming that in controls alveolar macrophages degrade between 5% and 100% of surfactant disaturated-phosphatidylcholine. Thus most of our conclusions remain valid independent of any assumption regarding the site of degradation of surfactant.

The present results agree with the view that, in ARDS, only a fraction of the lung is accessible to exogenous surfactant. In fact, the decrease of the alveolar pool of disaturated-phosphatidylcholine, the decrease of fluxes between tissue and alveoli and the decrease in the rate of synthesis can all be interpreted assuming that instilled surfactant reached only aerated lung sections. However, our data do not support the notion that these residual lung sections were normal, since the mean resident time of disaturated-phosphatidylcholine in lung parenchyma (MRT₁) was greater while the rate of recycling tended to be lower than in controls. The greater mean residence time of disaturated-phosphatidylcholine in lung tissue could be due to a number of factors, namely to a decreased ability to degrade surfactant components, to an increased reacylation of lyso phosphatidylcholine (favored by the increased availability of palmitate residues generated by phospholipase A₂, released by inflammatory cells), to a proliferation of type II cells, to the distribution of tracer to lung structures not pertaining to the surfactant system (i.e. infiltrating inflammatory cells), or to a combination of these phenomena [46]. The distribution of tracer to lung structures not pertaining to the surfactant system could explain the tendency towards a less efficient recycling of DSPC observed in patients with ARDS (figure 4).

**Conclusion**

Surfactant pool size is greatly diminished in ARDS compared to control, and surfactant kinetics is altered in ARDS resulting from a significantly reduced production rate and a significantly longer retention time in the 2nd (tissue) compartment.

The fact that the alveolar pool of disaturated-phosphatidylcholine can be estimated unambiguously is an important result of this work. In future studies this approach could be used to relate changes in surfactant turnover with time course and severity of ARDS or to evaluate the effect of different treatments (ventilation modes, inhaled or intravenous therapies) on surfactant metabolism.

**Abbreviations**

**ARDS** = acute respiratory distress syndrome

k₂₁ and k₁₂ = disaturated-phosphatidylcholine inter-conversion rate parameters,

k₀₁ and k₀₂ = disaturated-phosphatidylcholine irreversible losses,

u = labeled disaturated-phosphatidylcholine-palmitate injection into the accessible compartment.

M₁ = the alveolar pool of disaturated-phosphatidylcholine

M₂ = the tissue pool of disaturated-phosphatidylcholine

Mₜot= total disaturated-phosphatidylcholine pool

F₂₁, F₁₂, F₀₁, F₀₂ = disaturated-phosphatidylcholine inter-conversion and irreversible loss fluxes in compartment 1 (alveoli) and 2 (tissue)

P = *De novo* synthesis of disaturated-phosphatidylcholine

MRT₁ and MRT₂ = mean residence time of disaturated-phosphatidylcholine in compartment 1 (alveoli) and 2 (tissue)

**Competing interests**

The author(s) declare that they have no competing interests.
Authors’ contributions

PEC participated to the design and coordination of the study and drafted the manuscript. GMT, MC, CC performed the data modeling and analysis. CO and AV were responsible of the clinical conduction of the study. AG performed the mass spectrometry analysis. BA and VPC participated in the study design and helped to draft the manuscript.

Acknowledgements

We thank all patients who took part in the study and all the nurses for their precious contribution to the collection of the tracheal samples.

This study was funded by a grant from University of Padova, Italy and partially supported by Ministero dell’Universita e della Ricerca Scientifica, Italy.

References

1. Ware LB, Matthay MA: The Acute Respiratory Distress Syndrome. New Engl J Med 2000, 342:1334-1349.
2. Gastinoni L, Chiumello D, Cressoni M, Valenza F: Pulmonary computed tomography and adult respiratory distress syndrome, Swiss Med Wkly 2005, 135:169-174.
3. Gastinoni L, Pesenti A: The concept of baby lung. Intensive Care Med 2005, 31:776-784.
4. Frenling I, Guarin A, Seeger W, Pison U: Pulmonary surfactant: functions, abnormalities and therapeutic options. Intensive Care Med 2001, 27:1699-1717.
5. Haimata JJ, Papadakos Pj, Lachmann B: Surfactant therapy for acute lung injury/acute respiratory distress syndrome. Curr Opin Crit Care 2004, 10:18-22.
6. Panda AK, Nag K, Harbottle RR, Rodriguez-Capote K, Veldhuizen RA, Petersen NO, Possmayer F: Effect of acute lung injury on structure and function of pulmonary surfactant films. Am J Respir Cell Mol Biol 2004, 30:641-650.
7. Jobe AH: Phospholipid Metabolism and Turnover. In Fetal and Neonatal Physiology Edited by: Polin RA and Fox WW. Philadelphia, W. B. Saunders Company; 1992.
8. Malloy J, McCaig L, Veldhuizen RA, Yao LJ, Joseph M, Whitsett J, Lewis J: Alterations of endogenous surfactant system in septic adults. Am J Respir Crit Care Med 1997, 156:671-673.
9. Lewis JF, Ikegami M, Jobe A: Altered surfactant function and metabolism in rabbits with acute lung injury. J Appl Physiol 1990, 69:2303-2310.
10. Gurel O, Ikegami M, Chronescu ZC, Jobe AH: Macrophage and type II cell catabolism of SP-A and saturated phosphatidylcholine in mouse lungs. Am J Physiol Lung Cell Mol Physiol 2001, 280:L266-72.
11. Rider ED, Ikegami M, Jobe A: Intrapulmonary catabolism of surfactant-saturated phosphatidylcholine in rabbits. J Appl Physiol 1990, 69:1856-1862.
12. Atallah HL, Wu Y, Alaozi El-Azer M, Thouron F, Kounanvas K, Wolf C, Brochard L, Harf A, Delclaux C, Touqui L: Induction of type-IIA secretory phospholipase A2 in animal model of acute lung injury. Eur Respir J 2003, 21:1040-1045.
13. Fisher AB, Dodica C: Lysosomal-type PL2 and turnover of alveolar DPPC. Am J Physiol Lung Cell Mol Physiol 2001, 280:L748-54.
14. Bernard GR, Artiga A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, LeGall R, Morris A, Spragg R: The American-European Consensus Conference on ARDS: definitions, mechanisms, relevant outcomes, and clinical trials co-ordination. Am Rev Respir Dis 1994, 149:818-824.
15. Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959, 37:911-917.
16. Mason J, Nielsen-Bomgren J, Clements JA: Isolation of desaturated phosphatidylcholine with Osborne tetroxide. J Lipid Res 1976, 17:281-284.
17. Christie WW: The analysis of fatty acids. In Gas chromatography and lipids A practical guide Edited by: Christie WW. Ayr, Scotland, The Royal Society press; 1969. page 107.
18. Torresin M, Zimmermann LJI, Cogo PE, Cavicchioli P, Badon T, Giordano G, Zacchello F, Sauer PjJ, Carnielli VP: Exogenous Surfactant Kinetics in Infant Respiratory Distress Syndrome: a Novel Method with Stable Isotopes. Am J Respir Crit Care Med 2000, 161:1584-1589.
19. Pinto RA, Wright JR, Lesikar D, Benson BJ, Clements JA: Uptake of pulmonary surfactant protein C into adult rat lung lamellar bodies. J Appl Physiol 1993, 74:1005-1011.
20. Hallman M, Epstein BL, Gluck L: Analysis of labeling and clearance of lung surfactant phospholipids in rabbit. Evidence of bidirectional surfactant flux between lamellar bodies and alveolar lavage. J Clin Invest 1981, 68:742-751.
21. Baritussio A, Pettenazzo A, Benevento M, Alberti A, Gamba P: Surfactant protein C is recycled from the alveoli to the lamellar bodies. Am J Physiol Lung Cell Mol Physiol 1992, 263:L607-L611.
22. Cobelli C, Foster D, Toffolo G: In: Tracer Kinetics in Biomedical Research New York, USA, Plenum Publisher; 2000.
23. DiStefano Jl: Complete parameter bounds and quasidentifiability conditions for a class of unidentifiable linear system. BioSisc 1983, 65:107-117.
24. Barret PHR, Bell BM, Cobelli C, Golde H, Schmutzky A, Vicini P, Foster D: SAAMI: simulation, analysis and modeling software for tracer and pharmacokinetics studies. Metabolism 1998, 47:684-492.
25. Nakos G, Kitiouli EI, Tsangaris I, Lekka ME: Bronchoalveolar lavage fluid characteristics of early intermediate and late phases of ARDS. Alterations in leukocytes, proteins, PAF and surfactant components. Intensive Care Med 1998, 24:296-303.
26. Baudouin SV: Exogenous surfactant replacement in ARDS-one day, someday, or never? New Engl J Med 2004, 351:853-855.
27. Schmidt R, Meier U, Yabut-Perez M, Walmrath D, Grimminger F, Seeger W, Gunther A: Alteration of fatty acid profiles in different pulmonary surfactant phospholipids in acute respiratory distress syndrome and severe pneumonia. Am J Respir Crit Care Med 2001, 163:H95-100.
28. Anzueto A, Baughman RP, Guntupalli KK, Weg JG, Wiedemann HP, Raventos AA, Lemaire F, Long W, Zaccardelli DS, Pattishall EN: Aerosolized surfactants in adults with sepsis-induced acute respiratory distress syndrome. N Engl J Med 1996, 334:1417-21.
29. Gregory TJ, Steinberg K, Spragg R, Gadek JE, Hyers TM, Longmore WJ, Moylex MA, Cai GZ, Hite RD, Smith RM, Hudson LD, Crim C, Newton P, Mitchell BR, Gold AJ: Bovine surfactant therapy for patients with acute respiratory distress syndrome. Am J Respir Crit Care Med 1997, 155:1309-1315.
30. Spragg RG, Lewis JF, Walmrath HD, Johannigman J, Bellingan G, Laterre PF, Wite MC, Richards GA, Rippin G, Rathgeb F, Hafner D, Taut F, Seeger W: Effect of recombinant surfactant protein C-based surfactant on the acute respiratory distress syndrome. New Engl J Med 2004, 351:884-892.
31. Janssen DJ, Carnielli VP, Cogo PE, Seidner SR, Luijendijk IH, Wastimena DJL, Jobe AH, Zimmermann LJI: Surfactant phosphatidylcholine half-life and pool size measurements in premature baboons developing BPD. Pediatr Res 2002, 52:724-729.
32. Janssen DJ, Tibboel D, Carnielli VP, van Emmen E, Luijendijk IH, Wastimena JLD, Zimmermann LJI: Surfactant phosphatidylcholine pool size in human neonates with congenital diaphragmatic hernia requiring ECMO. J Pediatr 2003, 142:247-252.
33. Cogo PE, Carnielli VP, Bunt JEH, Badon T, Giordano G, Zacchello F, Sauer PjJ, Zimmermann LJI: Endogenous surfactant metabolism in critically ill infants measured with stable isotopes labeled fatty acids. Pediatr Res 1999, 45:242-246.
34. Cogo PE, Zimmermann LJI, Rossi F, Tormena F, Gamba P, Verlato G, Baritussio A, Carnielli VP: Surfactant synthesis and kinetics in infants with congenital diaphragmatic hernia. Am J Respir Crit Care Med 2002, 166:54-158.
35. Cogo PE, Zimmermann LJI, Pesavento R, Sacchetto E, Burighel A, Rossi F, Badon T, Verlato G, Carnielli VP: Surfactant Kinetics in Preterm Infants on mechanical ventilation who did and did not developed Bronchopulmonary Dysplasia (BPD). Crit Care Med 2003, 31:1532-1538.
36. Cogo PE, Zimmermann LJI, Meneghini L, Mainini N, Bordignon L, Suma V, Bufolo M, Carnielli VP: Pulmonary surfactant desaturated-phosphatidylcholine (DSPC) turnover and pool size in newborn infants with congenital diaphragmatic hernia (CDH). Pediatr Res 2003, 54:653-658.
37. Oyarzum MJ, Clements JA, Baritussio A: Ventilation enhances pulmonary alveolar clearance of radioactive dipalmityl phos-
phatidylcholine in liposomes. Am Rev Respir Dis 1980, 121:709-721.
38. Davis JM, Russ GA, Metlay L, Dickerson B, Greenspan BS: Short
term distribution kinetics of intratracheally administered
exogenous lung surfactant. Pediatr Res 1992, 31:445-450.
39. Ueda T, Ikegami M, Rider ED, Jobe A: Distribution of surfactant
and ventilation in surfactant-treated preterm lambs. J Appl
Physiol 1994, 76:45-55.
40. Rebello CM, Jobe AH, Eisele JW, Ikegami M: Alveolar and tissue
surfactant pool sizes in humans. Am J Respir Crit Care Med 1996,
154:623-628.
41. DiStefano JJ, Malone TK, Jang M: Comprehensive kinetics of thy-
roxine (T4) distribution and metabolism in blood and tissue
pools of the rat from only 6 blood samples: dominance of
large, slowly exchanging tissue pool. Endocrinology 1982,
111:108-117.
42. DiStefano JJ, Jang M, Malone TK, Broutman M: Comprehensive
kinetics of triiodothyronine (T3) production distribution and
metabolism in blood and tissue pools of the rat using optimi-
ised blood sampling protocol. Endocrinology 1982,
110:198-213.
43. Cobelli C, Toffolo G: Theoretical aspects and practical strate-
gies for the identification of unidentifiable compartmental
systems. In Identifiability of Parametric Models Edited by: Walter E.
Oxford, Pergamon Press; 1987:85-91.
44. Martini WZ, Chinkes DL, Barrow RE, Murphey ED, Wolfe RR: Lung
surfactant kinetics in conscious pigs. Am J Physiol 1999,
277:E187-95.
45. Young SL, Kremers SA, Apple JS, Crapo JD, Brumley GW: Rat lung
surfactant kinetics biochemical and morphometric correla-
tion. J Appl Physiol 1981, 51:248-253.
46. Fisher AB, Dodia C, Feinstein SI, Ho YS: Altered lung phospholi-
pid metabolism in mice with targeted deletion of lysosomal-
type phospholipase A2. J Lipid Res 2005, 46(6):1248-56.