Surfactant disaturated phosphatidylcholine kinetics in infants with bronchopulmonary dysplasia measured with stable isotopes and a two-compartment model

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Departments of 1Pediatrics and 2Information Engineering, University of Padova, Padova; 3Division of Neonatology, Ospedali Riuniti Ancona, Ancona, Italy; and 4Childhood Nutrition Research Centre, Institute of Child Health, University College London, London, United Kingdom

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Cogo, Paola E., Gianna Maria Toffolo, Antonina Gucciardi, Arianna Benetazzo, Claudio Cobelli, and Virgilio P. Carnielli. Surfactant disaturated phosphatidylcholine kinetics in infants with bronchopulmonary dysplasia measured with stable isotopes and a two-compartment model. J Appl Physiol 99: 323–329, 2005. First published March 23, 2005; doi:10.1152/japplphysiol.01423.2004.—We previously found a shorter surfactant disaturated phosphatidylcholine palmitate (DSPC-PA) half-life in infants with bronchopulmonary dysplasia (BPD) by using a single stable isotope tracer and simple formulas based on a one-exponential fit of the final portion of the enrichment decay curve. The aim of this study was to apply noncompartmental and compartmental analysis on the entire enrichment decay curve of DSPC-PA and to compare the kinetic data with our previous results. We analyzed 10 preterm newboms with BPD (gestational age 26 ± 0.6 wk, weight 777 ± 199 g) and 6 controls (gestational age 26 ± 1.4 wk, weight 787 ± 259 g). All took part in our previous study. Endotracheal 13C-labeled dipalmityl phosphatidylcholine was administered, and the 13C-enrichment of surfactant DSPC-PA was measured from serial tracheal aspirates by gas chromatography-mass spectrometry. Noncompartmental and compartmental models were numerically identified from the tracer-to-tracer ratio and kinetic parameters related to the accessible (pool accessible to sampling, likely to be the lung alveolar pool) and to the nonaccessible pools (pools not accessible to samplings, likely to be the intracellular storage pool) were estimated in the two study groups. Comparison was performed by Mann-Whitney test. A two-compartment model provided the most reliable assessment of DSPC-PA kinetics. In BPD vs. controls, mean ± SE residence time of DSPC-PA in the accessible was 17.5 ± 2.6 vs. 32.2 ± 6.4 h (P < 0.05), whereas it was 49.7 ± 3.5 vs. 54.4 ± 3.9 h (NS, not significant) in the nonaccessible pool; DSPC-PA recycling was 0.26 ± 0.05 vs. 0.43 ± 0.04% (NS), respectively. A two-compartment model of surfactant DSPC-PA kinetics allowed a thorough assessment of DSPC-PA kinetics, including masses, synthesis, and fluxes between pools. The most important findings of this study are that in BPD infants DSPC-PA loss from the alveolar pool was higher and recycling through the intracellular pool lower than in controls.

pulmonary surfactant; stable isotopes; phospholipids

BRONCHOPULMONARY DYSPLASIA (BPD) is a significant respiratory complication of current neonatal intensive care and a leading cause of neonatal morbidity and mortality during the first year of life. The factors implicated in its pathogenesis include arrested or delayed lung growth and acute lung injury induced by mechanical ventilation, oxygen toxicity, and inflammation (21).

Human and animal studies suggested that the amount and composition of pulmonary surfactant are altered (6, 23, 28) in BPD, but there is little information on surfactant kinetics. Pulmonary surfactant is a complex mixture of lipids and proteins that contains >90% by weight of phospholipids and four surfactant specific proteins. The vast majority of phospholipids is represented by disaturated phosphatidylcholine (DSPC), which is by far the most studied surfactant component (22). Surfactant DSPC kinetics has been measured in animal models by using radiolabels and two-compartment analysis or direct quantification of surfactant pools (18, 19, 29). In this setting, lungs can be completely lavaged and then minced at death to quantify surfactant alveolar and tissue pools, respectively (30). Animal studies, however, carry two major limitations: 1) significant differences in surfactant kinetics have been found depending on animal species, postnatal age, and degree of prematurity (16, 19, 24, 30); and 2) animal models may not entirely mimic the pathophysiology of human condition.

Studies with radiolabels and two-compartment models have never been performed in humans because of ethical constraints and inevitable limitations to sample collection. We recently described a new stable isotope approach to study surfactant DSPC kinetics in vivo in preterm and term infants with respiratory failure (32). We administered a tracer dose of stable isotope dipalmityl phosphatidylcholine (DPPC) through the endotracheal tube followed by sequential tracheal aspirate sampling over the study period. We measured the isotopic enrichments of disaturated phosphatidylcholine palmitate (DSPC-PA) from each tracheal aspirate, and we calculated DSPC-PA kinetics from the DSPC-PA enrichment curves over time (32). Our method was validated in a baboon animal model in which total DSPC pools and DSPC-PA half-life were similar when measured either in vivo with stable isotope or ex vivo from lung tissue (20). It is of note that sampling collection was more scattered in BPD baboons than in study infants (15, 32) and study time was shorter (20). Enrichment curves exhibited a monoexponential decay, and therefore kinetic calculation was straightforward.

Hence surfactant DSPC kinetic can be measured in vivo with stable isotopes. The technique inevitably provides less detailed

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measurements than those obtained from animal studies, in which lung tissue can be sampled, but it has the major advantage of studying human diseases in live subjects.

We also studied with the same technique BPD infants and gestational age- and study weight-matched controls (15). All DSPC-PA enrichment curves exhibited a biexponential decay, and we calculated DSPC-PA half-life and pool size by using only the final monoexponential part of the decay curve. We found a shorter DSPC-PA half-life and a larger pool size in BPD.

The aim of the present study was to verify whether our DSPC-PA kinetic results were confirmed by analyzing the entire biexponential decay curve with both noncompartmental and compartmental models in a subset of patients in whom these analyses were feasible (15).

Glossary

**Noncompartmental model parameters.**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_1$ (mg)</td>
<td>DSPC-PA mass in the accessible compartment</td>
</tr>
<tr>
<td>$R_a$ (mg/h)</td>
<td>Rate of DSPC-PA appearance in the accessible compartment</td>
</tr>
<tr>
<td>$\theta_1$ (h)</td>
<td>Mean residence time in the accessible compartment</td>
</tr>
<tr>
<td>$M_{RT}^{NC}$ (h)</td>
<td>Mean residence time in the system</td>
</tr>
<tr>
<td>$M_{tot}^{NC}$ (mg)</td>
<td>DSPC-PA mass in the system</td>
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</table>

**Compartmental model parameters.**

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<tr>
<td>$M_1$ (mg)</td>
<td>DSPC-PA mass in compartment 1</td>
</tr>
<tr>
<td>$M_2$ (mg)</td>
<td>DSPC-PA mass in compartment 2</td>
</tr>
<tr>
<td>$M_{tot}$ (mg)</td>
<td>DSPC-PA mass in the system</td>
</tr>
<tr>
<td>$k_{21}$, $k_{12}$, $k_{01}$ (h$^{-1}$)</td>
<td>Transfer rate parameters</td>
</tr>
<tr>
<td>$P$ (mg/h)</td>
<td>Rate of DSPC-PA de novo synthesis</td>
</tr>
<tr>
<td>$\theta_1$ (h)</td>
<td>Mean residence time in the accessible compartment</td>
</tr>
<tr>
<td>$M_{RT1}$ (h)</td>
<td>Mean residence time in the system of tracer moieties (entering the system from compartment 1)</td>
</tr>
<tr>
<td>$M_{RT2}$ (h)</td>
<td>Mean residence time in the system of endogenous particles (entering the system from compartment 2)</td>
</tr>
<tr>
<td>$F_{21}$, $F_{12}$, $F_{01}$ (mg/h)</td>
<td>Intercompartmental DSPC-PA fluxes</td>
</tr>
<tr>
<td>$R$ (%)</td>
<td>Recycling of DSPC-PA, equal to the ratio between $F_{21}$ and $F_{21} + F_{01}$ expressed as percentage</td>
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**MATERIALS AND METHODS**

**Patients.** We studied 16 preterm infants, 10 with BPD or at high risk of developing BPD and 6 with normal lungs or minimal lung disease, admitted to the Neonatal Intensive Care, University of Padova, Italy, who were part of our laboratory’s previous publication (15) and from whom a sufficient number of tracheal aspirates was available to perform the analyses. Clinical characteristics of the patients are reported in Table 1. The Ethics Committee, University Hospital of Padova, Italy approved the study, and written, informed consent was obtained from both parents.

**BPD infants.** All BPD infants had respiratory distress syndrome (RDS) at birth and were treated with exogenous surfactant and mechanical ventilation. BPD, or high likelihood of developing BPD, was diagnosed on the basis of lack of improvement of the respiratory condition after RDS, inspired oxygen fraction ($F_{O2}$) $>$0.40 after 3 wk of age, positive chest radiograms (29), and exclusion of infection. None of the study infants was treated with steroids after birth.

**Control infants.** Preterm infants with no or minimal lung disease requiring mechanical ventilation served as controls (Table 1). Inclusion criteria were 1) no sign of infection or of persistent ductus arteriosus at the time of the study, 2) normal chest X-ray, 3) $F_{O2}$ $<$ 30% at all time during the study period.

**Study design.** An intratracheal tracer dose of 5 mg/kg of uniformly $^{13}$C-palmitate ([U-$^{13}$C-PA]) DPPC was administered as described previously (14, 32) to all infants, to trace surfactant DSPC. Both palmitate (PA) moieties of the tracer molecule were uniformly labeled with the stable isotope carbon-13 (Martek Biosciences, Columbia, MD). Natural and synthetic DPPC have been shown to be metabolically indistinguishable when administered intratracheally in newborn rabbits (18), and it is universally accepted that no differences are to be expected between $^{12}$C and $^{13}$C isotopes during kinetic studies (34).

**Mode of ventilation.** Mode of ventilation was standardized with an inspiratory time of 0.3–0.5 s and a positive end-expiratory pressure of 2–4 cmH2O. Peak inspiratory pressure and $F_{O2}$ were adjusted so that the oxygen saturation was $>$88% but <96% and the arterial $PCO_2$ was between 40 and 50 Torr. Ventilator parameters (peak inspiratory pressure, $F_{O2}$, respiratory rate, mean airway pressure, and end-expiration pressure) were recorded before the start of the study and subsequently every 12 h.

Tracheal aspirates were collected before the administration of the tracer (time 0), at 3 h and 6 h, and then every 6 h until 72 h and every 12 h thereafter until extubation (32).

DSPC from tracheal aspirates was separated by thin-layer chromatography after treatment with osmium tetroxide (25, 33). DSPC fatty acids were derivatized as pentfluoroacetylbenzyl derivatives (5), extracted with hexane, and stored at $-$20°C until analysis (32). The enrichment of [U-$^{13}$C-PA]-DSPC from the tracheal aspirates was measured by gas chromatography-mass spectrometry (Voyager, Thermquest, Rodano, Milano, Italy) operating in negative ionization mode. Selective ion monitoring was carried out at mass-to-charge ratio 255, 256, 257, and 271, and results were expressed as tracer-to-tracee ratio (trt). The coefficient of variation for repeated samples (6 aliquots of a single tracheal aspirate, independently processed) was always $<$3%.

**Surfactant kinetic parameters.** The trt of DSPC-PA, namely the ratio between exogenously administered and endogenous DSPC-PA, was evaluated from ion current ratios (12). Surfactant DSPC-PA kinetics were quantified by applying noncompartmental and compartmental modeling approaches to trt data. In both cases, it was assumed...
that the constant endogenous steady state is not perturbed by the tracer administration.

The noncompartmental model (Fig. 1) (10) focuses on the accessible compartment, i.e., the alveolar pool where the endotracheal tracer is administered and where tracheal aspirates are sampled. On the basis of a multiexponential model fitted to DSPC-PA ttr

\[ ttr(t) = \sum_{i=1}^{N} A_i e^{-\alpha_i t} \] (1)

a number of noncompartmental parameters were calculated, related either to the accessible pool or to the system, namely:

DSPC-PA mass in the accessible compartment \( M_1 \) (mg)

\[ M_1 = \frac{D}{ttr(0)} = \frac{D}{\sum_{i=1}^{N} A_i \alpha_i} \] (2)

disappearance rate from the accessible pool \( R_a \) (mg/h)

\[ R_a = \frac{D}{\int_0^{ttr(t)} dr \sum_{i=1}^{N} A_i \alpha_i} \] (3)

mean residence times in the accessible compartment and in the system, respectively \( \theta_1 \) (h) and MRT\(_{NC} \) (h)

\[ \theta_1 = \frac{M_1}{R_a} \] (4)

\[ \text{MRT}_{NC} = \int_0^{ttr(t)} dt \sum_{i=1}^{N} A_i \alpha_i \] (5)

DSPC-PA mass in the system \( M_{tot}^{NC} \) (mg)

\[ M_{tot}^{NC} = R_a \cdot \text{MRT}_{NC} \] (6)

where \( D \) represents the DSPC-PA tracer dose (\( \mu \)mol).

The selection of the two-exponential model as the best representation of DSPC-PA data (see RESULTS) suggested the use of a two-compartment model to describe DSPC-PA kinetics (7). The model is shown in Fig. 2 where accessible compartment 1 likely reflects the alveolar pool and compartment 2 likely approximates the intracellular storage pool. DSPC-PA is synthesized intracellularly and then secreted into the alveolar surface, where it can either be recycled back to intracellular storage pool or be irreversibly lost.

**Tracer model equations**

\[ m_1(t) = -(k_{01} + k_{21})m_1(t) + k_{12}m_2(t) + u(t) \] \[ m_2(t) = -k_{21}m_2(t) + k_{12}m_1(t) \] \[ y(t) = \frac{m_1(t)}{M_1} \] (7)

where \( y \) is DSPC-PA tracer-to-tracee ratio measured in the accessible compartment; \( m_1 \) and \( m_2 \) (mg) are the amount of DSPC-PA tracer in compartments 1 and 2, respectively; \( k_{21}, k_{12}, k_{01} \) (h\(^{-1}\)) are transfer rate parameters; \( u \) is the tracer impulsive input into the accessible compartment; \( M_1 \) (mg) is the steady-state tracee mass in the accessible compartment; and overdots indicate derivatives with respect to time.

**Tracee model equations**

\[ M_1(t) = 0 = -(k_{01} + k_{21})M_1 + k_{12}M_2 \] \[ M_2(t) = 0 = -k_{12}M_2 + k_{21}M_1 + P \] (8)

where \( M_2 \) (mg) is the steady-state tracee mass in the compartment 2 and \( P \) (mg/h) is the rate of DSPC-PA de novo synthesis. The tracer model (Eq. 7) is a priori uniquely identifiable (9), i.e., unique values of unknown parameters \( k_{21}, k_{12}, k_{01} \), and \( M_1 \) can be identified from the data. Solution of tracee model (Eq. 8) provides an estimate of \( M_2 \) and \( P \). Hence, additional compartmental parameters can be defined to characterize the system, namely the total mass in the system, \( M_{tot} \) (mg); the mean residence time in the accessible compartment, \( \theta_1 \) (h), and in the system, \( \text{MRT}_1 \) (h), of tracer particles entering the system from compartment 1; the mean residence time in the system of the endogenous particles entering the system from compartment 2, \( \text{MRT}_2 \) (h); and the intercompartmental fluxes \( F_{21}, F_{12}, F_{01} \) (mg/h). Finally, the percentage \( R \) (%) of particles that recycle back after leaving the alveolar surface can be quantified from the ratio, expressed as percentage, between \( F_{21} \) and \( F_{21} + F_{01} \).

**Calculations.** One-, two-, and three-exponential models were fitted to DSPC-PA ttr data by using SAAMII software (4). 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 has been estimated a posteriori. The best exponential model was selected on the basis of criteria such as the evaluation of residual errors, the precision of parameter estimates, and the principle of parsimony (11).

The two-compartment model of Fig. 2 was fitted to DSPC-PA ttr data by using SAAMII (4). Weights were chosen as above.

**Statistics.** Values are expressed as means ± SE. Comparison between groups was performed by the Mann-Whitney test. A two-tailed probability value of <0.05 was considered statistically significant.

**RESULTS**

Of the 16 study infants, three BPD subjects (30%) and none of the control subjects died before hospital discharge. All three
deaths occurred several weeks after the study period. All six preterm infants with no lung disease were on mechanical ventilation for apnea of prematurity or because of insufficient respiratory drive. One had duodenal atresia, and five had RDS treated with exogenous surfactant at birth and subsequently recovered. No control infant was oxygen dependent at 36 wk of corrected gestational age. Clinical characteristics of the two study groups are reported in Table 1. Mean study weight and gestational age were comparable in the two groups, whereas ventilator parameters were significantly different, as expected by study design. BPD infants were significantly older at the time of the study \((P = 0.005)\). Six of 10 infants (60%) in the BPD group and 4 of 6 (66%) in the control group received prenatal steroids. No infant in either group received postnatal steroids as treatment for BPD before or at any time during the study.

The average ± SD time courses of DSPC-PA ttr, shown in Fig. 3, exhibit a rapid initial fall of the DSPC-PA ttr soon after the tracer administration followed by a slow long decay, with a faster decline in BPD patients compared with controls. In all subjects, parameters of the one- and of the two- but not of the three-exponential model were estimated with acceptable precision (Table 2). Conversely, the three-exponential model was too complex to be resolved from the available data (not shown). The ability of the one- and two-exponential model to reproduce the data is shown in Fig. 4, for one representative subject. The two-exponential model provided a reasonable fit, whereas the one-exponential model failed to reproduce the early portion of the decay curve. These results indicate that the two-exponential model provided the best representation of DSPC-PA kinetics. Values of its amplitudes (normalized to the tracer dose) and exponentials (Table 2) are not significantly different between the two groups; however, the amplitude of the first exponential tended to be higher and that of the second exponential to be lower in BPD patients compared with controls. The noncompartmental estimate of the mean residence time in the system was significantly lower in BPD patients compared with controls \((P < 0.025)\).

Compartmental model parameters of DSPC-PA kinetics are shown in Table 3. Values of DSPC-PA mass \(M_1\) and residence time \(\theta_1\) in the accessible compartment were the same when estimated by the two approaches (Tables 2 and 3). Similarly, the production rate \(P\) and the mean residence time of particles entering the system from the accessible compartment \(\text{MRT}_{\text{NC}}\)

### Table 2. Exponential model and noncompartmental parameters of DSPC kinetics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BPD</th>
<th>Control</th>
<th>BPD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A_1) mg (-1)</td>
<td>0.057 (27)*</td>
<td>0.092 (23)</td>
<td>0.518 (70)</td>
<td>0.242</td>
</tr>
<tr>
<td>(A_1) h (-1)</td>
<td>0.042 (11)</td>
<td>0.028 (20)</td>
<td>0.195 (51)</td>
<td>0.033</td>
</tr>
<tr>
<td>(A_2) mg (-1)</td>
<td>0.092 (23)</td>
<td>0.028 (20)</td>
<td>0.518 (70)</td>
<td>0.242</td>
</tr>
<tr>
<td>(A_2) h (-1)</td>
<td>0.042 (11)</td>
<td>0.028 (20)</td>
<td>0.195 (51)</td>
<td>0.033</td>
</tr>
<tr>
<td>(M_1) mg/kg</td>
<td>26.20</td>
<td>18.76</td>
<td>4.79</td>
<td>3.17</td>
</tr>
<tr>
<td>(M_{\text{NC}}) mg/kg</td>
<td>2.96</td>
<td>3.17</td>
<td>7.49</td>
<td>2.15</td>
</tr>
<tr>
<td>(R_{\text{a}}) mg (-1) kg (-1) h (-1)</td>
<td>0.394</td>
<td>0.311</td>
<td>0.9</td>
<td>0.58</td>
</tr>
<tr>
<td>(\theta_1) h</td>
<td>30.3</td>
<td>37.0</td>
<td>17.5</td>
<td>15.8</td>
</tr>
<tr>
<td>(\text{MRT}_{\text{NC}}) h</td>
<td>0.951†</td>
<td>0.497</td>
<td>6.9</td>
<td>4.9</td>
</tr>
<tr>
<td>(\text{MRT}_{\text{NC}}) h</td>
<td>30.3</td>
<td>37.0</td>
<td>17.5†</td>
<td>15.8</td>
</tr>
</tbody>
</table>

DSPC, disaturated phosphatidylcholine. See Glossary for definitions of parameters. \(A_1\) and \(A_2\) were normalized to the tracer dose; \(M_1\), \(M_{\text{NC}}\), and \(R_{\text{a}}\) to kg body wt. *Precision of parameter estimate, expressed as coefficient of variation (CV%), i.e., if \(p_i\) denotes the parameter estimate, one has CV% = SD(p_i)/p_i × 100. †Significantly different from controls.
Table 3. Compartmental model parameters of DSPC kinetics

<table>
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<tr>
<th>Parameter</th>
<th>BPD</th>
<th>Control</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$k_{011}$, h$^{-1}$</td>
<td>$k_{212}$, h$^{-1}$</td>
</tr>
<tr>
<td>--------------------</td>
<td>-------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Mean</td>
<td>0.167 (41)*</td>
<td>0.065 (30)</td>
</tr>
<tr>
<td>SE</td>
<td>0.022</td>
<td>0.017</td>
</tr>
</tbody>
</table>

See Glossary for definitions of parameters. $M_1$, $M_2$, $M_{tot}$, $P$, $F_{01}$, $F_{21}$, and $F_{12}$ were normalized to kg body wt. *Precision of parameter estimate, expressed as CV%, i.e., if p denotes the parameter estimate one has CV% = SD(p)/p * 100. †Significantly different from controls.

(Table 3) coincided with their noncompartmental counterparts, $R_a$ and $MRT_{NC}$ (Table 2). $MRT_1$ was significantly lower in BPD compared with normal subjects. Conversely, because endogenous particles are produced in the intracellular pool, the mean residence time in the system of endogenous particles is correctly estimated by $MRT_2$, which was higher than $MRT_1$ and not different between the two groups. Similarly, the compartmental estimate of the total mass in the system $M_{tot}$ (Table 3) was significantly higher than the noncompartmental estimate $M_{tot}^{NC}$ (Table 2). The disappearance flux from the alveolar compartment $F_{01}$ tended to be higher (27%) in BPD patients than in controls, whereas the flux from the alveolar to the intracellular pool, $F_{21}$, tended to be lower when expressed both in absolute terms (30% decrease) and as percentage of the total flux leaving the accessible compartment (40% decrease, $P = 0.07$ just failed to reach statistical significance).

In summary, the two-exponential model provide the best fit for DSPC-PA kinetic data. By applying compartmental analysis, we found that DSPC-PA $MRT_1$ was significantly shorter and that DSPC-PA recycling tended to be slower in BPD infants than in controls. DSPC-PA masses are expressed in milligrams per kilogram body weight of palmitic acid molecule in Tables 1 and 2. When converted to milligrams per kilogram body weight of whole-molecule of DSPC, results were the following: 1) accessible pool $8.9 \pm 1.8$ and $14.4 \pm 3.8$ mg/kg body wt in BPD vs. control infants; 2) nonaccessible pool $51.3 \pm 7.6$ vs. $37.6 \pm 9.3$ mg/kg; and 3) total DSPC pool $60 \pm 9$ vs. $49 \pm 11$ mg/kg in BPD compared with control infants, respectively. These differences were not statistically significant.

**DISCUSSION**

Our laboratory previously compared DSPC-PA kinetics in vivo in 13 preterm infants with BPD and in 8 infants with no or minimal lung disease (15). We found marked differences between BPD and controls, and alteration of surfactant kinetics in BPD did correlate with disease severity (15). We published these findings because of the novelty of the tracer technique and because of their clinical relevance. However, we did not make full use of all the available data, by simply relying on the final single-exponential decay of the curve. Here, we applied a more sophisticated analysis based on noncompartmental and compartmental modeling approaches to derive a more detailed picture of surfactant DSPC-PA kinetics from the entire enrichment time curve.

**Noncompartmental and compartmental analysis of DSPC-PA kinetics.** The most important advantage of applying this methodology was to obtain information on DSPC-PA kinetics in the accessible as well as in the nonaccessible portion of the system. In fact, the inability of the single-exponential model to reproduce the data in all study infants testifies to the presence of a compartmentalization of DSPC-PA at the subcellular level. The noncompartmental analysis was performed by adopting a two-exponential model (Fig. 4). The compartmental approach required to postulate the model structure of Fig. 2, where pool $1$ is the accessible pool, from where we sampled our specimens, likely to be the lung alveolar pool, whereas pool 2 likely approximates the intracellular storage pool. The noncompartmental and compartmental analysis yield equal values of parameters related to the accessible pool, such as $M_1$ and $\theta_1$. As regards system parameters, the $R_a$ in the accessible compartment correctly measures DSPC-PA production. Conversely, because DSPC-PA is produced in the nonaccessible pool (pool 2), the noncompartmental analysis underestimated the DSPC-PA mean residence time, and thus its total mass in the system (8). The compartmental analysis provided the most detailed and reliable evaluation of DSPC-PA kinetics, in terms of kinetic parameters such as mean residence times, pool masses, synthesis, and fluxes.

The most important results (Tables 2 and 3) can be summarized as follows: 1) the mean residence time in the system of particles entering the system from pool 1, recovered both via noncompartmental analysis (MRT$^{NC}$) and compartmental analysis (MTR$_1$), was significantly shorter in BPD infants than controls, whereas the mean residence time of endogenous particles, entering the system from compartment 2 (MTR$_2$), was not significantly different; 2) in BPD infants, alveolar DSPC-PA mass ($M_1$) was 15% of the total DSPC-PA mass in the system ($M_{tot}$), and it was 28% in the control group; 3) DSPC-PA flux from the alveolar to the intracellular pool ($F_{21}$) tended to be reduced in BPD; 4) DSPC-PA synthesis in the intracellular pool (P) was not impaired; and 5) total DSPC-PA loss from the system ($F_{01}$) tended to be higher in BPD infants, particularly when expressed per unit DSPC-PA mass ($k_{01}$); this was associated with a lower DSPC-PA recycling.

All these kinetic parameters have never been obtained before by using a single tracer. Surfactant DSPC or phosphatidylcholine synthesis, fluxes (turnover), and recycling were obtained in animals by means of two different radioactive labels and subsequent animal death and lung lavages at predetermined time points (3, 18, 29). More recently, we also administered simultaneously two stable isotope tracers in vivo in newborn infants with congenital diaphragmatic hernia to measure DSPC net synthesis. Our analysis was not bicompartimental, and...
Innovative Methodology

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therefore we could only estimate the DSPC net synthesis and the percentage of DSPC loss (either recycled or catabolized). No information regarding the kinetic of the two pools was obtained (13).

However, to apply the modeling analysis proposed here, an appropriate number of samples (in the present study, 14 data time points corresponding to a 5-day period) needs to be collected. This obviously increased the complexity of the study.

Comparison with animal data. DSPC kinetic was reported by Seidner and colleagues (29) in a BPD baboon animal model. Surfactant DSPC-PA synthesis and half-life were measured by means of radioactive tracers and alveolar and lung tissue DSPC pools by direct measurements. They found that alveolar DSPC pool size was 12% of the total lung pool and that preterm BPD baboons had higher DSPC-PA synthesis but lower DSPC-PA secretion than control animals. This caused intracellular DSPC accumulation (30). In our BPD infants, we estimated that the alveolar DSPC-PA pool size (pool 1) was 14% of the total mass pools (pool 1 + pool 2) (Fig. 2), with the total mass being 15% higher in BPD than in controls. Conversely, we did not find decreased DSPC-PA synthesis or secretion, whereas DSPC-PA flux from pool 1 to pool 2 tended to be reduced compared with control infants. This could be consistent with an impaired DSPC-PA recycling. Whether reduced recycling is caused by an impairment of the DSPC-PA pathway or it is related to alterations of other surfactant components, such as surfactant-specific proteins, cannot be assessed in this study, and it needs to be determined (1, 2, 26).

We also measured DSPC-PA pool size at 14 days of age in 123-day-old mechanically ventilated baboons (20). We found that total DSPC-PA pool was 90 mg/kg. It is noteworthy that animal data provide the total amount of DSPC in the lungs, whereas with tracer kinetics in vivo we estimated DSPC-PA mass. The total DSPC pool in our BPD infants was 60 mg/kg, which is one-third less compared with our baboon data (30). This difference in DSPC pool size could be due to 1) animal species (baboon vs. humans), 2) clinical management, including nutrition regimen or various degree of mechanical ventilation (17), 3) larger amount of exogenous surfactant administered in Seidner’s study but not in our patients, or 4) different severity of the disease.

Mean DSPC-PA half-life reported by Seidner et al. (30) was 48 h, remarkably longer compared with the mean residence time of 17 h found in our BPD infants. DSPC clearance was found to be longer in rabbits when multiple doses of bovine surfactant were administered (29), clearly showing that the larger the amount of DSPC given with the tracer, the longer was the DSPC half-life. Seidner administered [14C]DPPC together with a treatment dose of 100 mg/kg of exogenous surfactant (31), whereas our BPD infants received only 5 mg/kg of DPPC. Thus the larger DSPC dose in Seidner’s study probably accounts for the longer DSPC half-life (31).

Comparison with our previous kinetic parameters. To compare our findings with the “standard” DSPC kinetics, i.e., DSPC-PA kinetic parameters previously reported by us, we recalculated mean ± SE DSPC-PA half-life and pool size in the same subset of patients with the standard method. It is noteworthy that, in the case of a single exponential model, half-life, divided by the ln(2), i.e., 1.44, measure the mean residence time in the system. We found that the mean residence time estimated with the standard method was remarkably similar to the mean residence time in the system of tracer particles, entering the system from the accessible compartment, estimated from the biexponential fit, via either the noncompartmental or the compartmental analysis (MRT<sub>NC</sub> = MRT<sub>1</sub>) both in BPD (17.5 ± 2.6 and 15.3 ± 3.5 h compartmental vs. standard method, respectively) and in control group (32.2 ± 6.4 and 29.5 ± 5.4 h, respectively). However, the standard mean residence time was consistently longer than that of endogenous DSPC-PA entering the system from compartment 2, which is correctly measured by the compartmental parameter MRT<sub>2</sub>. Total DSPC-PA pool sizes were 115 ± 26 vs. 60 ± 9 mg/kg and 57 ± 9 vs. 49 ± 11 mg/kg in BPD and controls, respectively (standard vs. bicompartamental model). These higher values of total DSPC-PA pool size obtained with the standard method could be a consequence of the poor fit of the first part of the decay enrichment curve, thus implying that the standard method is less accurate, particularly in BPD subjects in whom the first exponential has a higher amplitude compared with the second exponential. Nevertheless, as previously discussed, we validated our standard kinetic method against a baboon animal model (20). Total DSPC pool size measurements in vivo (or volume of distribution) via the standard method produced figures comparable to the direct determination on the tissue plus alveolar wash. Limitations of the DSPC pool size estimates in vivo in humans were discussed by our group in a previous publication (15). It is noteworthy that the biochemical determination of DSPC lung tissue after lung lavage at death, which is regarded as the “reference method,” may overestimate the “actual” surfactant-related DSPC, because of the contribution of DSPC from tissue components other than surfactant, and from cell membranes (inflammatory and type I and II cells and vascular and stromal cells). Overestimation of lung tissue DSPC can also be caused by incomplete washout of alveolar surfactant, which may occur more often when sick lungs are lavaged. Conversely, the DSPC pool size estimated with the two-compartment analysis is likely to be associated with the actual metabolically active surfactant, where the tracer is distributed.

Limitations of the study. Our kinetic results, although in line with previous human and animal studies, cannot be conclusive because of the limited number of patients and the wide standard deviation of the kinetic variables. BPD is a complex disease with a variable degree of severity, and it is conceivable that DSPC-PA kinetics varies with the degree of lung disease. Therefore, further studies are needed to confirm our data in a larger group of infants.

In conclusion, in this study, we performed a detailed non-compartmental and compartmental analysis of DSPC-PA kinetics, using gas chromatography-mass spectrometry tracer-to-tracee ratio data. When compared with the standard parameters of DSPC-PA kinetics previously reported, our results indicate that the standard method provides a reasonable estimate of the mean residence time of the tracer, but not of endogenous DSPC-PA, whereas DSPC-PA pool size was higher with the standard method compared with the two-compartment model analysis. The major advantage of the novel analysis here proposed is that with a single tracer we were able to calculate DSPC-PA masses, synthesis, fluxes, catabolism, and recycling.
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