

## Surfactant kinetics in ventilated infants

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### Animal studies had limitations

Surfactant kinetics have been measured in various animal models using radiolabeled DSPC and two-compartment analysis, or by direct quantification of surfactant pools.<sup>[1-3]</sup> For example, alveolar lavage was used to quantify the alveolar pool of <sup>14</sup>C-saturated phosphatidylcholine (Sat PC) in preterm baboons; lungs were then minced at animal sacrifice to quantify the tissue pool of Sat PC.<sup>[4]</sup> However, animal studies had several limitations:

- significant differences in surfactant kinetics exist, depending on the animal species studied, postnatal age and degree of prematurity;<sup>[3-6]</sup>
- animal models do not always mimic the human pathophysiology. Some models (e.g. prematurity, surfactant lavage) appear to be more 'physiologic' than others (e.g. injury induced by oleic acid or teratogenic chemicals).

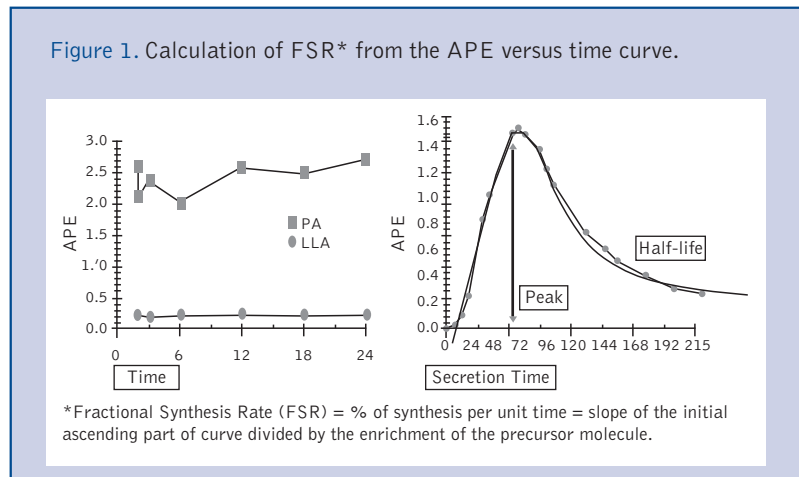
### Information on surfactant metabolism and kinetics in humans is limited

Until recently, only limited data were available on surfactant metabolism in human neonates. Limited information can be obtained from the measurement of the amount of surfactant components from serial tracheal aspirates. Surfactant half-life was extrapolated from the disappearance of PG relative to sphingomyelin from serial airway samples collected after exogenous surfactant administration.<sup>[7]</sup>

### Two main research objectives established

In 1996–1998, the paucity of information about surfactant kinetics in human neonates prompted us to design novel studies with the following main objectives.

- **Measurement of endogenous surfactant synthesis** using non-radioactive, naturally-occurring stable isotopes as surfactant precursors in biosynthesis studies. We wanted to study surfactant synthesis in relation to several clinical conditions such as prenatal factors, different diseases and disease phases, and finally in relation to different medical interventions. In most cases, <sup>13</sup>C-glucose, <sup>13</sup>C-leucine or <sup>13</sup>C-palmitate were used as surfactant precursors and administered by a continuous intravenous infusion. FSR, the percentage of surfactant component synthesised per unit time, was calculated by dividing the slope of the initial ascending part of the APE versus time curve (for tracheal aspirates) by <sup>13</sup>C-enrichment of the precursor molecule in plasma (*Figure 1*).
- **Evaluation of the pharmacokinetics of exogenous surfactants** labeled with tracers. In this series, stable isotope-labeled DPPC was prepared and mixed with exogenous surfactant before intratracheal administration. After administration of this intratracheal tracer, the APE versus time curve showed, in most cases, a biexponential decay such that DPPC pool size and half-life could be calculated.



## Endogenous surfactant synthesis studies

In the first study to record data about endogenous surfactant production and turnover in preterm infants,<sup>[8]</sup> six infants were given a continuous intravenous infusion of <sup>13</sup>C-glucose 4.2 mg/kg/min as a precursor for surfactant; an FSR of 2.7% and a half-life of 113 hours were calculated for surfactant PC palmitate.<sup>[8]</sup>

This is the first in a series of 'endogenous' studies in which endogenous surfactant DSPC synthesis has been studied using different metabolic precursors (glucose or lipids) and in different clinical circumstances.

Studies were presented on CDH as an example of the information that can be obtained from the application of stable isotope technology to surfactant metabolism and kinetics. In the first study on CDH, DSPC synthesis and kinetics were evaluated in 12 infants with CDH and five control subjects given a 24-hour intravenous infusion of <sup>13</sup>C-palmitate.<sup>[9]</sup> No significant differences between CDH patients and controls were noted regarding DSPC FSR (22% vs 17%), ST (8.3 vs 8.5 hours), peak time (51.9 vs 51.0 hours), and half-life (59.0 vs 43.0 hours) [Figure 2].<sup>[9]</sup> The first study indicated that DSPC synthesis in CDH was rather similar to that observed in control subjects. However, it should be noted that FSR values can be misleading because FSR represents a percentage of an unknown amount of product. A high FSR of a small amount of product (small pool size) represents a relatively small absolute synthesis, whereas a relatively small FSR of a large pool reflects a significant synthesis. ASR is therefore a much more meaningful parameter of synthesis that can be calculated from the FSR and the pool size. Another study was therefore conducted in 13 CDH infants and eight controls given an intratracheal dose of <sup>13</sup>C-palmitate-DPPC in order to obtain information on the DSPC pool size and DSPC half-life.<sup>[10]</sup> In this trial, CDH patients had a significantly lower apparent DSPC pool size (34 vs 57 mg/kg,  $p=0.02$ ) and significantly shorter half-life (24 vs 53 hours,  $p=0.01$ ) versus controls (Figure 3).<sup>[10]</sup>

## Surfactant synthesis is preserved in CDH

In a third CDH study, the endogenous and the exogenous isotope tracers were administered simultaneously in 10 CDH infants and six controls: that is, study participants were given intratracheal <sup>13</sup>C-DPPC and intravenous <sup>2</sup>H-palmitate.<sup>[11]</sup> Overall, no significant differences were evident between CDH patients and controls regarding FSR and apparent pool size. Because of the double tracer administration in this particular

Figure 2. DSPC synthesis and kinetics in CDH neonates and controls.<sup>[9]</sup>

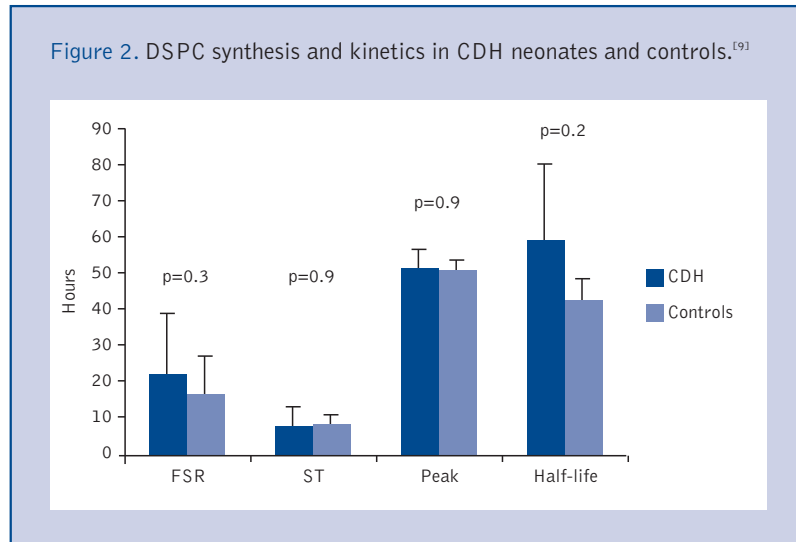
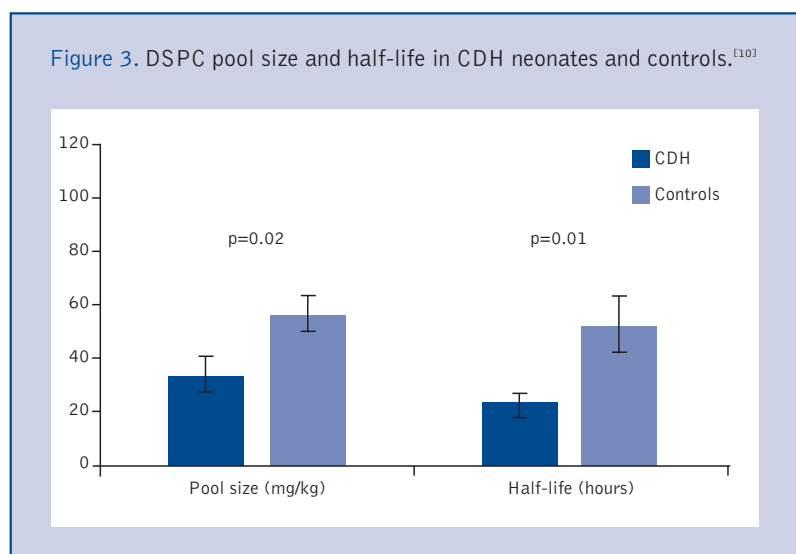


Figure 3. DSPC pool size and half-life in CDH neonates and controls.<sup>[10]</sup>



study the ASR could be calculated (Table 1). This study was the first to describe the measurement of DSPC net synthesis in humans, and concluded that surfactant synthesis was preserved in CDH patients.<sup>[11]</sup>

Table 1. DSPC kinetics in CDH patients and controls<sup>[10]</sup>

	CDH (n=10)	Controls (n=6)	p value
FSR (%/day)	21	15	0.06
Apparent pool size (mg/kg)	36.7	58.5	0.07
ASR (net synthesis; mg/kg/day)	8.6	8.1	0.70

### Studies evaluating DSPC kinetics

Simultaneous measurement of DSPC kinetics and synthesis from plasma glucose and free fatty acid in preterm infants revealed an approximately equal contribution to DSPC secretion time, FSR, peak time and half-life from each of the two different precursors (data on file; manuscript submitted for publication). Further studies are being conducted to ascertain whether dietary intervention during parenteral nutrition can affect surfactant synthesis in preterm infants and can influence the contribution of glucose or lipids to DSPC synthesis.

In a separate study in infants with BPD, an exogenous tracer was used to demonstrate that dexamethasone significantly ( $p=0.037$ ) prolonged DSPC half-life from approximately 20 to 35 hours, although the latter value remained about half that in age-matched controls not receiving steroid therapy.

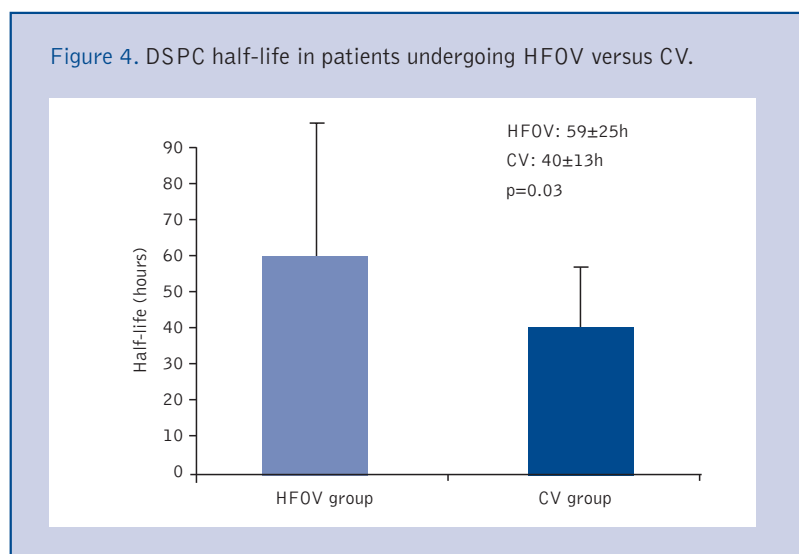
We are also conducting a series of studies with the aim of understanding the impact of the ventilation style on surfactant metabolism. Preliminary data from one of these studies show that HFOV is associated with a significantly longer DSPC half-life than CV (59 vs 40 hours,  $p=0.03$ ) [Figure 4]. This finding might explain the lower amount of exogenous surfactant used in randomised studies of neonates undergoing HFOV versus CV.

### SP-B kinetics can be determined

SP-B and DSPC kinetics and synthesis from plasma <sup>13</sup>C-valine and <sup>2</sup>H-palmitate were measured simultaneously in term neonates.<sup>[12]</sup> The mean FSR of SP-B (30%/day) was greater than that of DSPC, whereas ST, peak time and half-life were markedly shorter for SP-B than DSPC (Figure 5). This preliminary study clearly showed that SP-B kinetics can be measured *in vivo* with stable-isotope technology.<sup>[12]</sup>

### Exogenous surfactant kinetic data are emerging

The effects of dosing and disease on the pharmacokinetics of exogenous surfactant are of interest to us. For example, in one trial in progress, an exogenous surfactant dose of 100 or 200 mg/kg was administered to preterm infants with RDS. The half-life after the first dose was significantly longer in the high-dose than low-dose group ( $\approx 50$  vs 25 hours;  $p=0.018$ ); after the second dose, the half-life was longer in both groups ( $\approx 55$  vs 35 hours) and, although the between-group difference remained statistically

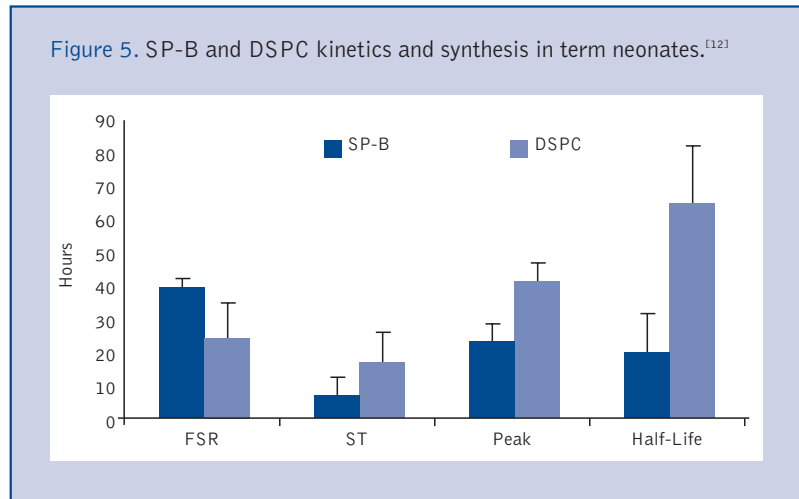


significant ( $p=0.02$ ), it was smaller. These findings clearly indicate that the dose and timing of surfactant administration influence surfactant kinetics.

### DSPC distribution is altered in ARDS

A novel model has been designed in which a single intratracheal tracer is administered to infants, and tracer-to-tracee decay-curve data are obtained over time.<sup>[13]</sup> Using this model, important information about DSPC kinetics can be derived, assuming bicompartamental distribution of DSPC in the alveoli

and pulmonary tissue: that is, data about DSPC fluxes between the alveolar and intracellular compartments or pools can be obtained, as can data about DSPC synthesis. Thus, a major difference was identified between surfactant status in patients with ARDS and controls: alveolar and lung-parenchymal pools of DSPC were markedly reduced in ARDS, compared with controls.



### Reduced surfactant may be linked to extubation failure

A recent trial was designed to determine whether the amount of surfactant present in the lungs of infants recovering from RDS correlates with the extubation failures or with worsening respiratory function after extubation. Inclusion criteria comprised birthweight <1500 g, age <13 days, RDS requiring MV within 24 hours of birth, and extubation between 3 and 10 days of life; strict extubation criteria were employed. Preliminary results showed a tendency ( $p=0.07$ ) towards reduced DSPC pool size in preterm infants who failed extubation, compared with infants who were successfully extubated.

### Conclusions

Novel stable-isotope technology permits research into the following areas:

- surfactant (i.e. lipid and protein) kinetics;
- pathophysiological aspects of altered surfactant metabolism;
- the pharmacokinetics of exogenous surfactants in various lung diseases;
- the effects of genes, hormones and nutrition on endogenous surfactant metabolism.

The novel information that will be obtained will likely be of great importance and clinical relevance for the management of newborn infants and adults with respiratory disorders.

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