Anal Bioanal Chem (2014) 406:6225–6233
DOI 10.1007/s00216-014-8014-2

RESEARCH PAPER

Surfactant protein C metabolism in human infants and adult patients by stable isotope tracer and mass spectrometry

Manuela Simonato · Aldo Baritussio · Barbara Pioselli · Carlo Ori · Silvia Catinella · Virgilio P. Carnielli · Paola E. Cogo

Received: 9 May 2014 / Revised: 29 June 2014 / Accepted: 30 June 2014 / Published online: 16 August 2014

Abstract Surfactant protein C (SP-C) is deemed as the surfactant protein most specifically expressed in type II alveolar epithelial cells and plays an important role in surfactant function. SP-C turnover in humans and its meaning in the clinical context have never been approached. In this study, we used mass spectrometry to investigate SP-C turnover in humans. We studied four infants and eight adults requiring mechanical ventilation. All patients had no lung disease. Patients received a 24-h continuous infusion of ^{13}C-leucine as precursor of SP-C, and serial tracheal aspirates and plasma samples were obtained every 6 h till 48 h. SP-C was isolated from tracheal aspirates by sorbent-phase chromatography. ^{13}C-leucine SP-C enrichment could be successfully measured in three infant and in four adult samples by using mass spectrometry coupled with a gas chromatographer. Median SP-C fractional synthesis rate, secretion time, and peak time were 15.7 (14.1–27.5) %/day, 6.0 (4.7–11.5) h, and 24 (20–27) h. In conclusion, this study shows that it is feasible to accurately determine SP-C turnover in humans by stable isotopes.

Keywords Surfactant protein C · Lung surfactant · Isotope · Mass spectrometry

Electronic supplementary material The online version of this article (doi:10.1007/s00216-014-8014-2) contains supplementary material, which is available to authorized users.

M. Simonato (✉)
Women's and Children's Health Department, University of Padova, 35128 Padova, Italy
e-mail: manuela.simonato@unipd.it

A. Baritussio
Department of Medicine, University of Padova, 35128 Padova, Italy

B. Pioselli · S. Catinella
Corporate Preclinical R&D, Analytics and Early Formulations Department, Chiesi Farmaceutici, 43122 Parma, Italy

C. Ori
Department of Medicine, Anesthesiology and Intensive Care, University of Padova, 35128 Padova, Italy

V. P. Carnielli
Division of Neonatology, Department of Mother and Child Health, Ospedale Salesi-Azienda Ospedaliero Universitaria Ospedali Riuniti, 60123 Ancona, Italy

V. P. Carnielli
Department of Medical Sciences, Polytechnic University of Marche, 60121 Ancona, Italy

P. E. Cogo
Pediatric Cardiac Anesthesia/Intensive Care Unit, Department of Pediatric Cardiology and Cardiac Surgery, Bambino Gesù Children's Hospital, 00165 Rome, Italy

Introduction

Surfactant protein C (SP-C) is composed, depending on the species, of 33–35 amino acid residues and is one of the most hydrophobic proteins in the proteome, accounting for 4 % of phospholipids by weight in large aggregate of premature infants [1, 2]. Its extreme hydrophobicity derives from the presence of a hydrophobic membrane spanning domain, a hydrophobic N-terminal domain [3], and two palmitoyl residues bound to cysteines 5 and 6 [4].

SP-C is expressed primarily in type II alveolar epithelial cells and is synthesized as a precursor protein (proSP-C) that is converted, by a series of processing steps, into mature SP-C. Mature SP-C is stored in the lamellar bodies and finally secreted into the alveolar spaces.

SP-C is the most specifically expressed surfactant protein in type II cells [5]. As a result, measuring the levels of SP-C could be used to gauge the ability of the lung epithelial cells to produce surfactant.

SP-C knockout mice grow normally without any apparent pulmonary abnormalities. They have normal alveolar structure, lamellar bodies, tubular myelin, and surfactant saturated phosphatidylcholine pool size, indicating that SP-C has no role in the intracellular biosynthesis, packaging, and secretion of surfactant. However, SP-C null mice generate a surface film that is unstable at low lung volumes [6]. Furthermore, SP-C has a role in maintenance of lung function during hyperoxia [7]. Lu and co-workers have recently observed a reduction in SP-C expression in infants dying from respiratory distress syndrome who were ventilated at elevated oxygen concentrations [8].

Mutations in the SP-C gene have been associated with familiar interstitial lung disease and adult pulmonary fibrosis [9, 10]. A mutation in the SP-C gene has been recently identified in a young woman with bronchiectasis and severe respiratory insufficiency [11–13]. Marked heterogeneity in severity, age of presentation, and time of presentation of the pulmonary disease may be influenced by genetic background as reported in a study with SP-C knockout mice where a strain differences was observed [14].

Current data on the turnover of SP-C are available only in animals [15–17]. Stable isotope tracer has been used by our group to trace surfactant protein B (SP-B) metabolism [18, 19], therefore the goal of this study was to demonstrate the feasibility of measuring SP-C metabolism in infants and adults using ^{13}C-leucine as tracer.

Methods

Materials

Acetic acid, hydrochloric acid, HPLC grade chloroform, hexane, and methanol were obtained from Carlo Erba Reagents (Milan, Italy). Analytical grade trifluoroacetic acid and trifluoroacetic anhydride were purchased from Sigma Chemicals (Milan, Italy). [1^{13}C]L-leucine with isotopic purity of >99 % was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). All solvent used were tested for aminoacids contamination.

Subjects

SP-C kinetics was studied in four infants and eight adults who were admitted to the neonatal intensive care unit and to the intensive care unit, City Hospital of Padova, respectively. All patients had no lung disease and needed mechanical ventilation after major surgery or for neurological failure.

The local ethics committee approved the protocol and informed consent was obtained from all patients or, in the case of infants, from both parents. These patients were also part of two larger studies done to assess the turnover of SP-B [20, 21].

Study design

All infants received a 24-h constant i.v. infusion of 2 mg/kg/h of 1^{13}C-leucine dissolved in saline. Adults received a 24-h constant i.v. infusion of 1 g 1^{13}C-leucine (about 42 mg/h independently of body weight).

Baseline EDTA-blood and tracheal aspirates (TA) samples were obtained prior to the administration of the isotope. To establish the isotopic enrichment of leucine at plateau, blood samples were collected every 6 h during the infusion period (24 h). TAs were obtained every 6 h until 72 h. Blood was centrifuged at 1,300×*g* for 10 min; plasma was isolated and stored at −80 °C until analysis. TAs were performed according to a standardized procedure [22] and centrifuged at 400×*g* for 10 min; supernatant was stored at −80 °C.

Isolation of SP-C from TA

Preliminary phosphorous assay [23] was made on the TA sample in order to avoid overloading of solid-phase extraction columns. Aliquots of TAs containing 200 μg of phospholipids were extracted according to a modified Bligh and Dyer method [19]. The organic phase was dried under nitrogen, resuspended in 1 ml of chloroform and applied to a Bond Elute NH2 column previously activated with 3–5 ml of chloroform. Fractions were eluted with 3 ml of the following chloroform/methanol/glacial acetic acid mixtures: 20:1:0, 9:1:0, 4:1:0, 4:1:0.025, 3:2:0, 1:4:0, and 1:9:0 [18].

Determination of SP-C fractions purity

Western blot analysis performed previously by our group on a bronchoalveolar lavage sample containing 150 μg of phospholipids revealed that SP-C started to be recovered with the mixture 3:2:0 but most of the protein eluted with the fraction 1:4:0 (antibody anti SP-C was provided by Altana Pharma AG, Konstanz, Germany) [18]. Selective elution of SP-C in fractions 1:4:0 and 1:9:0, derived from SPE elutions (see above), was further confirmed by high-resolution mass spectrometry. To that end, 4:1:0, 4:1:0.025, 3:2:0, 1:4:0, and 1:9:0 fractions were individually loaded onto a C4 reversed-phase LC column (3.9×15 mm, Deltapak C4, 100 A, Waters, MA, USA) installed on a nano HPLC Dionex Ultimate 3000 interfaced with a Orbitrap LTQ XL (Thermo Fisher Scientific, MA, USA). Isocratic chromatographic conditions were adopted and a constant flow of chloroform/methanol was used. High-resolution direct analysis of intact SP-B and SP-C was performed in positive ionization mode based on the deconvolution of the most abundant charged states of the intact proteins. Were considered the triple, fourth, and fifth

charge states for SP-C and eleventh, tenth, ninth, eighth, and seventh for SP-B.

SP-C kinetics

To study the incorporation of ^{13}C leucine into SP-C, 1:4:0 and 1:9:0 fractions were pooled together, evaporated to dryness, and hydrolysed to free amino acids for 24 h at 100 °C in 500 μl of 6 N HCl.

Amino acids from acid hydrolysis were then derivatized into their oxazolinone derivatives [24]. To determine leucine plasma enrichment, 100 μl of plasma were deproteinized with sulphosalicilic acid (6 % *w*/*v*) and plasma amino acids were derivatized according to Husek [25].

Both free plasma leucine and SP-C leucine enrichments were measured on a 6890N gas chromatographer coupled to an Agilent 5973i mass spectrometer (Agilent Technologies Italia SpA, Cernusco sul Naviglio, Italy).

Oxazolinone derivatives were analyzed by negative ion chemical ionization. Leucine was separated on a 50 m× 0.20 mm×0.33 μm Ultra 2 fused silica capillary column (Agilent technologies, Italia, SpA). Oven temperature was programmed for 1 min at 80 °C, increased from 80 to 180 °C at 6 °C min^{-1}, increased from 180 to 280 °C at 30 °C min^{-1}, and held at 280 °C for 3 min. The ion monitored for leucine was *m*/*z* 209 while for 1^{13}C-leucine it was *m*/*z* 210. Results were expressed as mole percent excess (MPE) referring to a calibration curve for 1^{13}C-leucine ranging from 0 to 5.23 MPE for oxazolinone derivatives and from 0 to 9.94 MPE for ethyl chloroformate derivatives.

Data analysis

All kinetic measurements were performed between 6 and 24 h assuming a 1^{13}C-leucine steady state. The assumption was based on the following considerations: (1) in all patients, plasma ^{13}C-leucine enrichment reached steady state within 6 h from the start of the isotope infusion; (2) the slope of the enrichment curve over time did not deviate significantly from zero between 6 and 24 h.

Fractional synthesis rate (FSR), secretion time (ST), and peak time (PT) were calculated as previously reported [21, 18]. Briefly, FSR was calculated by dividing the slope of the linear increase of the enrichment of SP-C by the plasma steady-state enrichment of free ^{13}C-leucine.

ST of SP-C was defined as the time-lag between the start of the precursor infusion and the appearance of the enriched product. The time-lag was calculated by plotting the regression line for the linear increasing part of the enrichment versus time curve and extrapolating it to baseline enrichment.

PT was the time required to reach maximum enrichment of SP-C 1^{13}C-leucine. Kinetic variables were expressed as individual values and as median and interquartile range or as mean and standard deviation as appropriate.

Results

Patients

We observed no side effects after the infusion of ^{13}C-leucine. Clinical details of the study patients are showed in Table 1. All patients were clinically stable during the study period. No one had lung disease or signs of infection. Five patients required mechanical ventilation for more than 48 h for major surgery (two adults and three infants). Three patients needed mechanical ventilation for impaired neurological function (two adults and one infant), and four adult patients were on spontaneous breathing via a tracheostomy.

SP-C purity

TAs were centrifuged to sediment cells and the chloroformic extract of the resulting supernatant was fractionated with Bond Elute NH_2 columns as previously described [18]. Fractions eluted with mixtures of choloform:methanol:acetic acid 4:1:0, 4:1:0.025 (containing SP-B), and 1:4:0 and 1:9:0 (containing SP-C) were analyzed with LC-HRMS analysis. HR-MS spectra of intact (not subjected to hydrolysis) SP-B and SP-C are shown in Figs. 1 and 2, respectively. The LC column and the mobile phase choice were optimized for highly sensitive and selective SP-B and SP-C detection. A reversed-phase C4 column was chosen based on the size and on the high hydrophobicity of the two proteins since the aim of the investigation was the analysis of the two surfactant intact proteins. Isocratic conditions successfully resolved SP-B and SP-C within the TA samples and the purity of the peaks could be easily estimated. The most critical electrospray source parameters affecting ionization were the sheath and auxiliary gases which were experimentally tuned according to the signal intensity obtained for each protein molecule. A specific tune page was then set for selective detection of each proteins. For the purpose of the method, we aimed at determining the purity of the fractionated TA samples with respect to SP-B and SP-C. There are no standard reference surfactant protein B and C which would enable the determination of the analytical limit of detection of surfactant proteins within TA, and therefore a relative semiquantitation based on detectable peaks area was performed proving the application of the optimized parameters for each protein.

Within fractions 1:4:0 and 1:9:0, we did not find any measurable amount of SP-B, which is primarily eluted in fractions 4:1:0 (>85 %) and 4:1:0.025 (15 %).

Table 1 Clinical characteristics of adult (panel a) and infants (panel b) patients

Panel a						
Patients	Sex	Weight (kg)	Age (year)	Intubation (day)	Ventilator mode	Main diagnosis
1	F	100	55	25	CPAP	Cerebral aneurysm rupture
2	F	50	26	3	PCV	Head trauma
3	M	90	42	25	SB	Cerebral aneurysm rupture
4	M	70	67	70	SB	Cerebral aneurysm rupture
5	F	60	76	2	PCV	Cerebral aneurysm rupture
6	M	55	18	32	SB	Drug addiction overdose
7	F	50	24	3	SB	Polytrauma
8	M	75	75	2	PCV	Subdural hematoma
Panel b						
Patients	Sex	Weight (kg)	GA (week)	Age (day)	Intubation (day)	Ventilator mode	Main diagnosis
9	M	5.4	39	44	3	SIMV	Bladder exstrophy
10	M	3.1	36	26	2	SIMV	Hydrocephalous
11	F	2.5	37	29	3	SIMV	Neuromiopathy
12	F	3.2	39	1	1	SIMV	Diaphragm plication

Age age at the beginning of the study, *GA* gestational age, *intubation* days of intubation before the beginning of the study. Ventilator mode: *PCV* pressure controlled ventilation, *CPAP* continuous positive airway pressure, *SB* spontaneous breathing via tracheostomy, *SIMV* synchronized intermittent mandatory ventilation

Kinetic data

In 4 of the 12 patients, the signal of ^{13}C leucine SP-C was too weak (signal-to-noise ratio for *m*/*z* 210 below 300) for determining the MPE ratio. Moreover, in one infant, two of the TA samples collected had an insufficient amount of SP-C. Therefore, leucine-SP-C kinetics was successfully measured only in three of the four infants and in four of the eight adults.

Plasma leucine enrichment achieved the plateau within 6 h from the start of the study (Electronic Supplementary Material Fig. S1). Isotopic equilibrium was maintained during the remaining 18 h of ^{13}C-leucine infusion. Mean enrichment value was 6.2±1.0 MPE in infants and 3.8±1.2 MPE in adults. The mean value was 6.3±1.2 and 5.1±0.8 MPE in infants and adults where SP-C kinetics was successfully measured, whereas it was 5.9 (patient 12) and 3.1±0.2 (adults) MPE in those patients where SP-C kinetics could not be measurable.

We found a coefficient of variation <3 % for each plasma and TA sample measured in triplicate. Figure 3 reports SP-C isotopic enrichment curves in our patients together with SP-B curves obtained in the same patients in previous studies [18, 21]. As shown in Fig. 3, incorporation of ^{13}C leucine into SP-C increased until 20–25 h after the start of isotope infusion with a PT of 24 h [20–27] like in SP-B. The maximum enrichment of ^{13}C leucine was higher for SP-B than for SP-C. Labeled SP-C appeared in the alveoli in 6.0 (4.7–11.5) h with a time-lag similar of that of SP-B. Both hydrophobic surfactant proteins were turned over within 48 h. The FSR of these proteins was rapid and we calculate that 15.7 (14.1–27.5) % of SP-C was newly synthesized each day; this value was about one third of that of SP-B. All kinetics results are shown in Table 2.

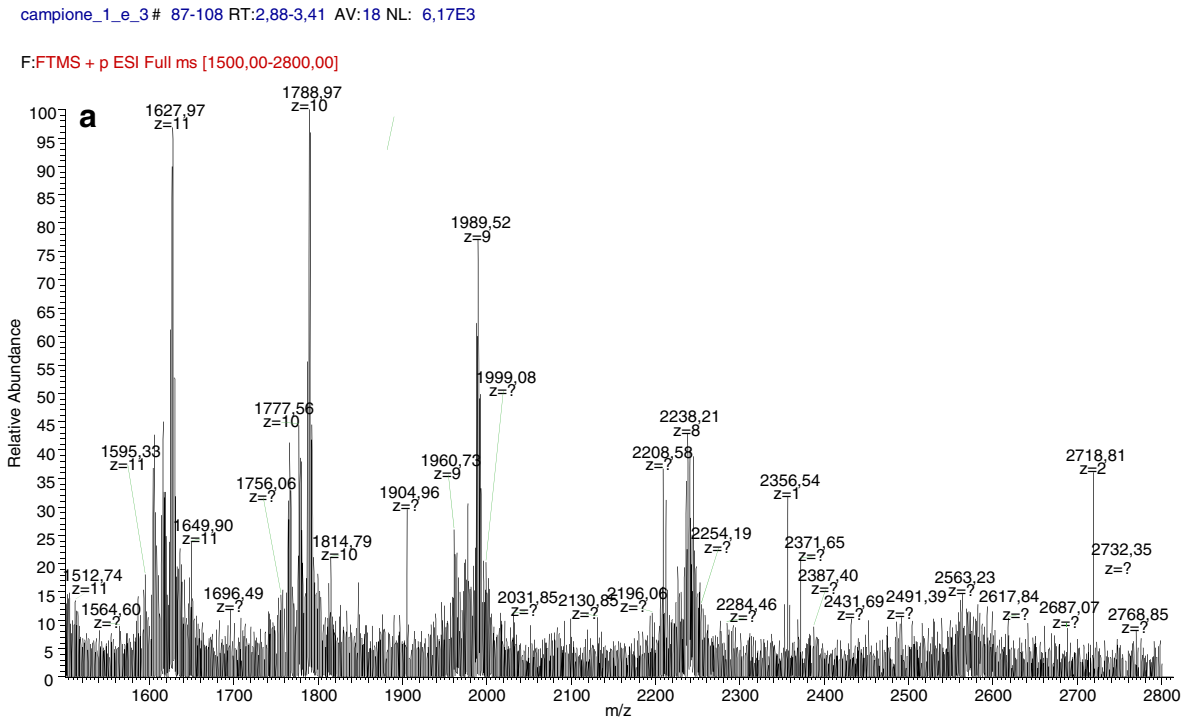
Discussion

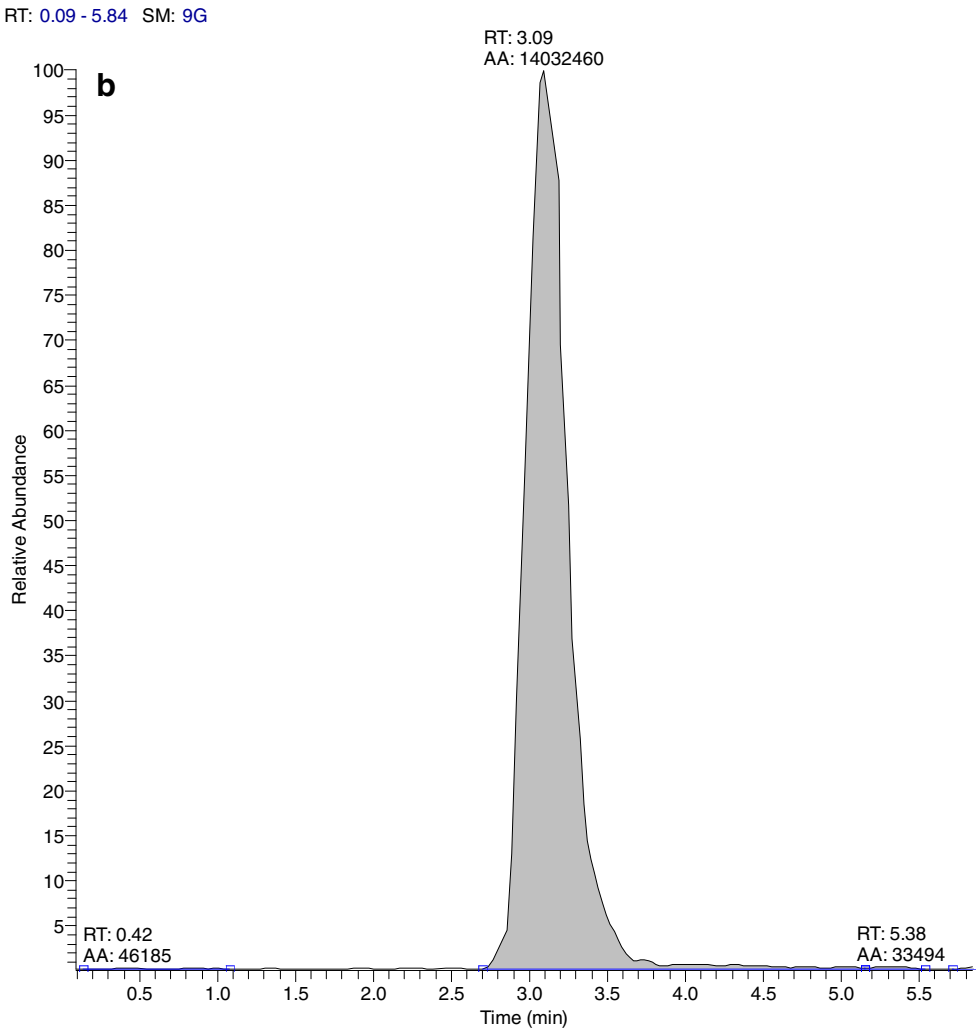
Stable isotopes have been used to study the turnover of surfactant components [18, 26]. We now add the turnover of SP-C with the hope that this may help to clarify the pathophysiology of lung disease.

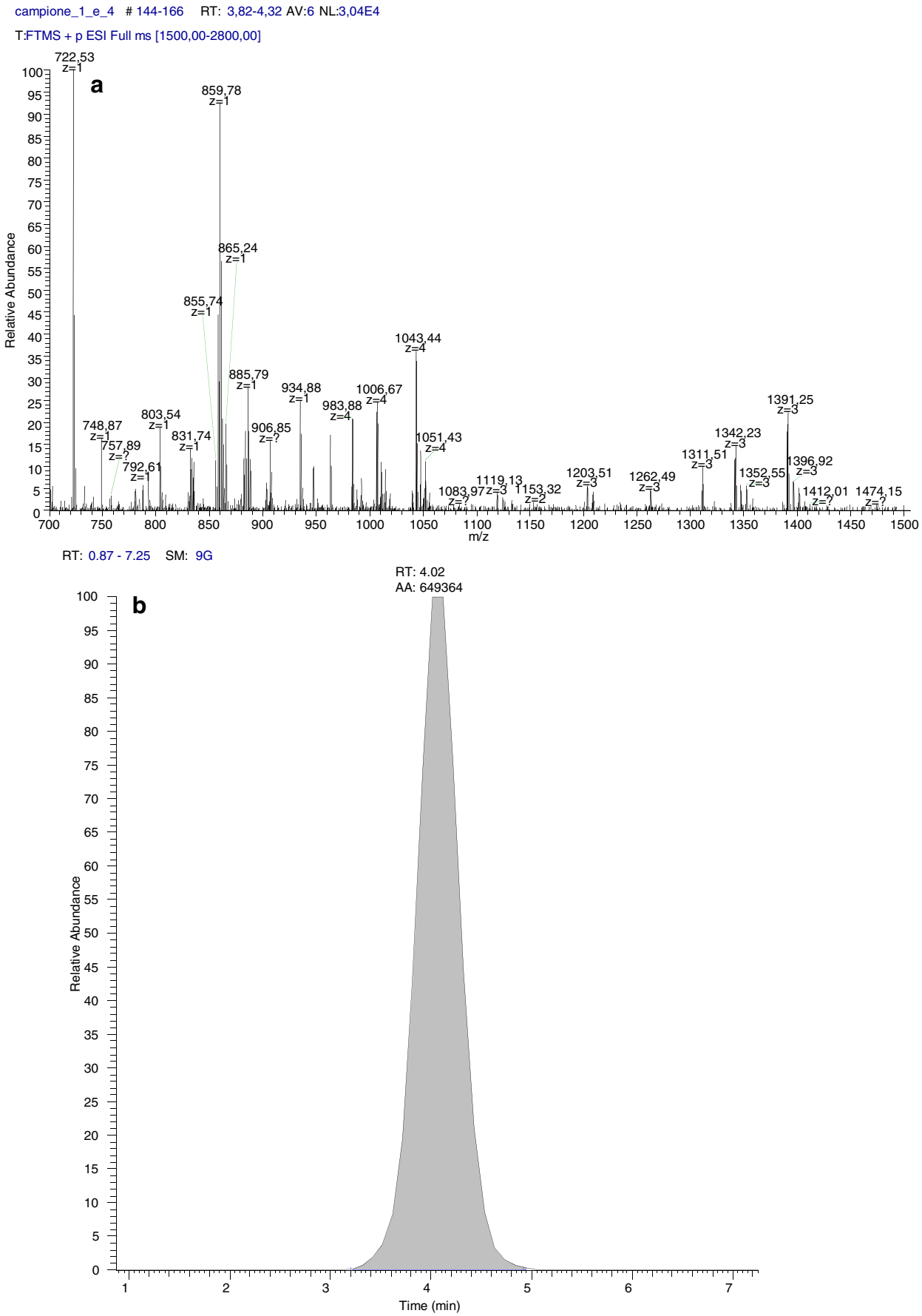
SP-C accounts for 2.1 and 1.3 % of total phospholipids in surfactant large aggregates obtained from term infants and adults with no lung diseases, while it is barely detectable in samples obtained from premature infants within the first days of life increasing to 4 % over subsequent week of life [2]. Existing evidence indicates that SP-C may not be critical for respiratory adaptation at birth but is important for normal postnatal lung function in special conditions like hyperoxia, infection, or lung immaturity [27, 8, 7, 28].

Studying the renewal of SP-C in newborn and adult subjects, we found that FSR, ST, and PT assumed a rather wide spectrum of values, possibly due to the heterogeneity of patients. Since all these patients have also been studied for SP-B and disaturated phosphatidylcholine (DSPC) kinetics [20, 21], we compared the turnover of SP-B and SP-C. Our study shows that labeled SP-C appears in the lungs after the same time lag of SP-B. This implies that these proteins have similar secretion pathways. We could also observe that SP-C turnover rate is lower compared to the one found for SP-B in

Fig. 1 *Panel A*, HRMS spectrum of human SP-B homodimer at 3.09 min. *Panel B*, extracted ion chromatogram of [SP-B+10H]10+ charge state ▶


campione_1_e_3 # 87-108 RT:2,88-3,41 AV:18 NL: 6,17E3
F:FTMS + p ESI Full ms [1500,00-2800,00]
a
Relative Abundance
m/z
1627,97 z=11
1788,97 z=10
1989,52 z=9
1999,08 z=?
1777,56 z=10
1595,33 z=11
1960,73 z=9
1756,06 z=?
1904,96 z=?
1649,90 z=11
1814,79 z=10
1512,74 z=11
1564,60 z=?
1696,49 z=?
2031,85 z=?
2130,85 z=?
2196,06 z=?
2208,58 z=?
2238,21 z=8
2254,19 z=?
2284,46 z=?
2356,54 z=1
2371,65 z=?
2387,40 z=?
2431,69 z=?
2491,39 z=?
2563,23 z=?
2617,84 z=?
2687,07 z=?
2718,81 z=2
2732,35 z=?
2768,85 z=?


RT: 0.09 - 5.84 SM: 9G
b
Relative Abundance
Time (min)
RT: 3.09
AA: 14032460
RT: 0.42
AA: 46185
RT: 5.38
AA: 33494


campione_1_e_4 # 144-166 RT: 3,82-4,32 AV:6 NL:3,04E4
T:FTMS + p ESI Full ms [1500,00-2800,00]
a
Relative Abundance
m/z
722,53 z=1
859,78 z=1
865,24 z=1
855,74 z=1
1043,44 z=4
885,79 z=1
934,88 z=1
1006,67 z=4
983,88 z=4
803,54 z=1
748,87 z=1
906,85 z=?
831,74 z=1
757,89 z=?
1051,43 z=4
792,61 z=1
1391,25 z=3
1342,23 z=3
1311,51 z=3
1396,92 z=3
1352,55 z=3
1203,51 z=3
1262,49 z=3
1119,13 z=3
1083,97 z=?
1153,32 z=2
1412,01 z=?
1474,15 z=?
RT: 0.87 - 7.25 SM: 9G
RT: 4.02
AA: 649364
b
Relative Abundance
Time (min)

◀ **Fig. 2** *Panel A*, HRMS spectrum of human dipalmitoyl SP-C at 4. 02 min. *Panel B*, extracted ion chromatogram of [SP-C+4H]4+ charge state

adult and in infant patients (Table 2). SP-C FSR ranged from 22 to 55 % and from 6 to 31 % of that of SP-B in adults and in infants, respectively. Only patient 4 renewed the SP-C pool faster than that of SP-B (66.3 vs. 47.0 %/day). Kristensen et al. has recently found that proteins of the same functional class seems to have similar synthesis and degradation rates [29], so, since SP-B and SP-C are packaged together into the lamellar bodies, differences in turnover

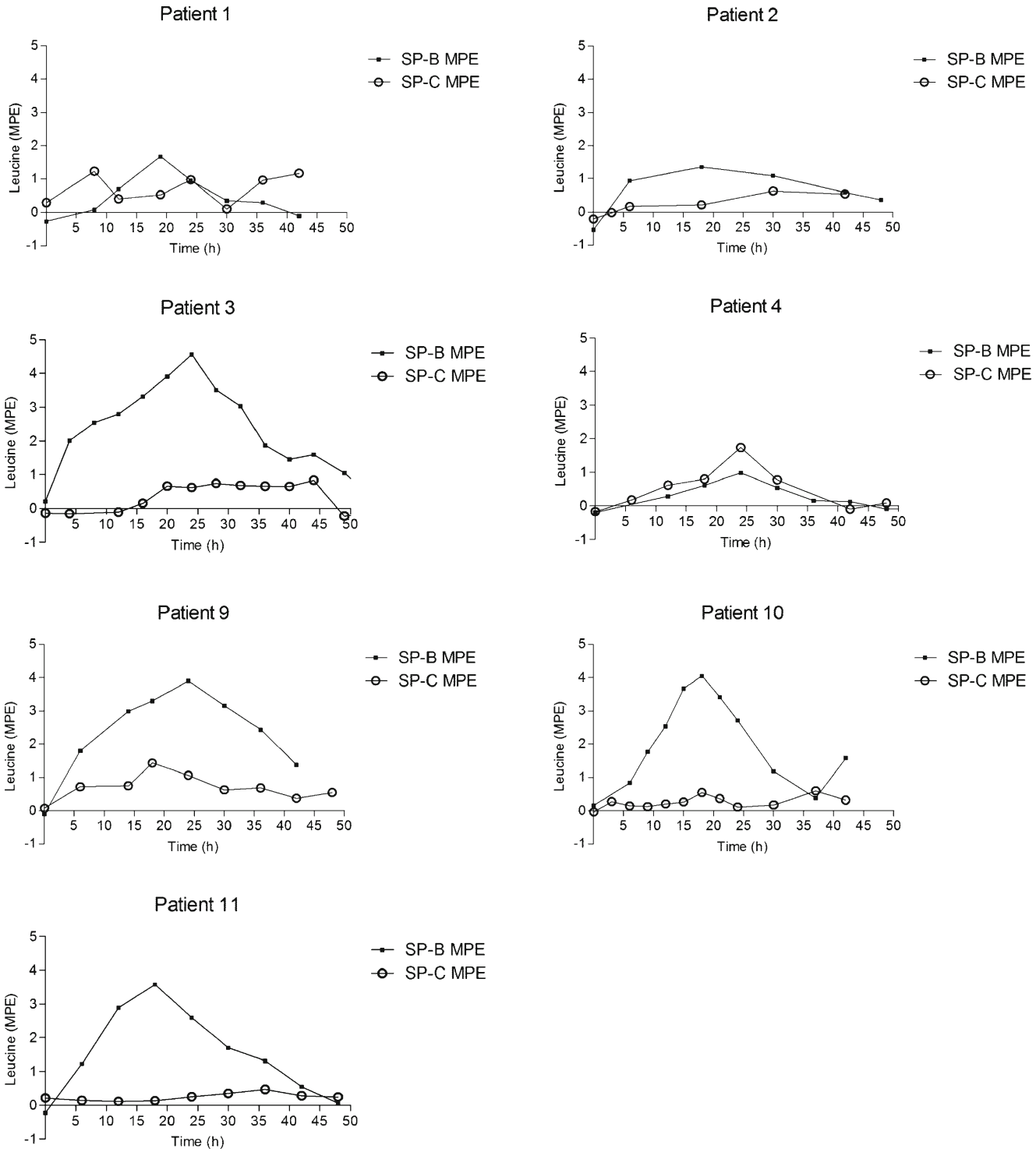


Fig. 3 ^{13}C leucine SP-C (*empty circles*) and ^{13}C leucine SP-B (*black squares*) enrichment curves in adult patients (patients 1 to 4), and infants (patients 9, 10, and 11) with no lung diseases receiving a 24-h infusion of ^{13}C-leucine. SP-B data are from [18, 21]. *MPE* mole percent excess

Table 2 Kinetics data [18, 21]

	SP-C			SP-B		
Patients	FSR (%/day)	ST (h)	PT (h)	FSR (%/day)	ST (h)	PT (h)
1	16.8	4.6	24	52.5	6.4	19
2	11.9	2.4	30	53.7	1.8	18
3	31.1	13.0	28	56.9	11.8	24
4	66.3	4.8	24	47.0	7.1	24
9	14.6	21.7	18	47.0	10.3	18
10	13.9	7.1	18	87.9	10.3	18
11	5.1	2.9	36	88.4	1.1	18
Median	15.7	6.0	24	53.1	8.7	19
(IQR)	(14.1–27.5)	(4.7–11.5)	(20–27)	(48.4–56.1)	(6.6–10.3)	(18–23)

FSR fractional synthesis rate, *ST* secretion time, *PT* peak time

may be due to a different destiny of these two proteins after secretion into the alveolar spaces.

There are no data on the turnover of SP-C in humans and previous works on the clearance of SP-C administered into the trachea of mice and rabbits have given different results. During short-time experiments (0–4 h) in which labeled surfactant was administered into the trachea of newborn rabbits, Baritussio et al. [15] found that SP-C is lost from the airways and appears in the lamellar bodies more rapidly than DSPC. On the opposite in studies performed over tens of hours, Ikegami and colleagues reported similar rates of removal of DSPC and SP-C from adult rabbit lungs (half life of about 11 h), whereas in mice, SP-C was cleared at a slower rate (half life 28 h) than DSPC (half life 12 h) [17]. One possible explanation of these discrepancies is that short studies may reflect events happening in the alveolar spaces, while longer studies tend to reflect events happening in the whole lung.

In this study, the percentage of failures in obtaining workable enrichment curves was 25 % in infants, and 50 % in adults. Interestingly, the adults with lower plasma 1^{13}C-leucine enrichment were the ones in whom SP-C kinetics could not be assessed. Considering the dose of isotope, infants received 2 mg/kg/h of 1^{13}C-leucine, whereas adults received a fixed dose not calculated on their body weight (0.6± 0.2 mg/kg/h). Thus, it is possible that a different isotope dosing could allow measuring SP-C kinetics in more adult subjects. Alternatively, the use of a different isotope, like [5,5,5-$^{2}H_3$] leucine, could improve the detection of the enriched signal. The triply-labeled amino acid have a lower background signal (given by the natural abundance) than the single-labeled one, leading to a better precision of the leucine isotope ratio measurement. Limits of this study were also the fact that some TAs did not contain enough material for analysis and the low frequency of sampling that could be increased at early time points. To improve the SPs recovery from TAs, we have now standardized the suctioning technique and decreased the dilution of the sample. It is noteworthy that lung fluid obtained by bronchoalveolar lavage (BAL) reflects better the composition of the alveolar surface and could contain a higher amount of surfactant protein [30] than that obtained by TA that collects surfactant present in the upper ways. Unfortunately, BAL is an invasive procedure and is not acceptable for ethical reason to repeat a BAL procedure every 6 h as per requested by our study design. On the other hand, tracheal aspiration is a less invasive technique and is often performed several times daily as part of the care of intubated patients. Moreover, the surfactant pool accessible by tracheal aspiration has been recently demonstrated to have a similar phospholipids composition and to be on isotopic equilibrium with the alveolar surfactant pool [31], thus making the tracheal aspirate samples a reliable substitute of bronchoalveolar lavage sample for kinetic study.

Further studies are needed to optimize the dose or the type of the isotope and to better characterize protein recovery.

In summary, we showed that it is feasible to measure SP-C kinetics in vivo in humans by stable isotope techniques. Future studies will focus on the relationship between the nature and extent of selected lung disease and the turnover of SP-C to better understand the role of this unique lung protein.

References

1. Beers MF, Mulugeta S (2005) Surfactant protein C biosynthesis and its emerging role in conformational lung disease. Annu Rev Physiol 67:663–696. doi:10.1146/annurev.physiol.67.040403.101937
2. Ballard PL, Merrill JD, Godinez RI, Godinez MH, Truog WE, Ballard RA (2003) Surfactant protein profile of pulmonary surfactant in premature infants. Am J Respir Crit Care Med 168(9):1123–1128. doi:10.1164/rccm.200304-479OC200304-479OC

3. Plasencia I, Rivas L, Casals C, Keough KM, Perez-Gil J (2001) Intrinsic structural differences in the N-terminal segment of pulmonary surfactant protein SP-C from different species. Comp Biochem Physiol A Mol Integr Physiol 129 (1):129–139
4. ten Brinke A, van Golde LM, Batenburg JJ (2002) Palmitoylation and processing of the lipopeptide surfactant protein C. Biochim Biophys Acta 1583(3):253–265
5. Orgeig S, Hiemstra PS, Veldhuizen EJ, Casals C, Clark HW, Haczku A, Knudsen L, Possmayer F (2010) Recent advances in alveolar biology: evolution and function of alveolar proteins. Respir Physiol Neurobiol 173:S43–S54. doi:10.1016/j.resp.2010.04.023
6. Glasser SW, Burhans MS, Korfhagen TR, Na CL, Sly PD, Ross GF, Ikegami M, Whitsett JA (2001) Altered stability of pulmonary surfactant in SP-C-deficient mice. Proc Natl Acad Sci U S A 98(11): 6366–6371. doi:10.1073/pnas.101500298
7. Ikegami M, Weaver TE, Conkright JJ, Sly PD, Ross GF, Whitsett JA, Glasser SW (2002) Deficiency of SP-B reveals protective role of SP-C during oxygen lung injury. J Appl Physiol 92(2):519–526. doi:10.1152/japplphysiol.00459.2001
8. Lu H, Li W, Shao G, Wang H (2012) Expression of SP-C and Ki67 in lungs of preterm infants dying from respiratory distress syndrome. Eur J Histochem 56(3):e35
9. Wang WJ, Mulugeta S, Russo SJ, Beers MF (2003) Deletion of exon 4 from human surfactant protein C results in aggresome formation and generation of a dominant negative. J Cell Sci 116(Pt 4):683–692
10. van Moorsel CH, van Oosterhout MF, Barlo NP, de Jong PA, van der Vis JJ, Ruven HJ, van Es HW, van den Bosch JM, Grutters JC (2010) Surfactant protein C mutations are the basis of a significant portion of adult familial pulmonary fibrosis in a Dutch cohort. Am J Respir Crit Care Med 182(11):1419–1425. doi:10.1164/rccm.200906-0953OC
11. Salerno T, Peca D, Rossi FP, Menchini L, Danhaive O, Cutrera R (2013) Bronchiectasis and severe respiratory insufficiency associated with a new surfactant protein C mutation. Acta Paediatr 102(1):e1–e2. doi:10.1111/apa.12043
12. Stevens PA, Pettenazzo A, Brasch F, Mulugeta S, Baritussio A, Ochs M, Morrison L, Russo SJ, Beers MF (2005) Nonspecific interstitial pneumonia, alveolar proteinosis, and abnormal proprotein trafficking resulting from a spontaneous mutation in the surfactant protein C gene. Pediatr Res 57(1):89–98. doi:10.1203/01.PDR.0000147567.02473.5A
13. Wolters PJ, Collard HR, Jones KD (2013) Pathogenesis of Idiopathic Pulmonary Fibrosis. Annu Rev Pathol. doi:10.1146/annurev-pathol-012513-104706
14. Glasser SW, Detmer EA, Ikegami M, Na CL, Stahlman MT, Whitsett JA (2003) Pneumonitis and emphysema in sp-C gene targeted mice. J Biol Chem 278(16):14291–14298. doi:10.1074/jbc.M210909200
15. Baritussio A, Pettenazzo A, Benevento M, Alberti A, Gamba P (1992) Surfactant protein C is recycled from the alveoli to the lamellar bodies. Am J Physiol 263(5 Pt 1):L607–L611
16. Baritussio A, Alberti A, Quaglino D, Pettenazzo A, Dalzoppo D, Sartori L, Pasquali-Ronchetti I (1994) SP-A, SP-B, and SP-C in surfactant subtypes around birth: reexamination of alveolar life cycle of surfactant. Am J Physiol 266(4 Pt 1):L436–L447
17. Ikegami M, Horowitz AD, Whitsett JA, Jobe AH (1998) Clearance of SP-C and recombinant SP-C in vivo and in vitro. Am J Physiol 274(6 Pt 1):L933–L939
18. Cogo P, Baritussio A, Rosso F, Gucciardi A, Moretti V, Badon T, Duner E, Zimmernann L, Carnielli VP (2005) Surfactant-associated protein B kinetics in vivo in newborn infants by stable isotopes. Pediatr Res 57(4):519–522. doi:10.1203/01.PDR.0000155755.27716.04
19. Simonato M, Baritussio A, Vedovelli L, Lamonica G, Carnielli VP, Cogo PE (2012) Surfactant protein B amount and kinetics in newborn infants: an optimized procedure. J Mass Spectrom 47(11):1415–1419. doi:10.1002/jms.3065
20. Cogo PE, Simonato M, Danhaive O, Verlato G, Cobellis G, Savignoni F, Peca D, Baritussio A, Carnielli VP (2013) Impaired surfactant protein B synthesis in infants with congenital diaphragmatic hernia. Eur Respir J 41(3):677–682. doi:10.1183/09031936.00032212
21. Simonato M, Baritussio A, Ori C, Vedovelli L, Rossi S, Dalla Massara L, Rizzi S, Carnielli VP, Cogo PE (2011) Disaturated-phosphatidylcholine and surfactant protein-B turnover in human acute lung injury and in control patients. Respir Res 12:36. doi:10.1186/1465-9921-12-36
22. Torresin M, Zimmermann LJ, Cogo PE, Cavicchioli P, Badon T, Giordano G, Zacchello F, Sauer PJ, Carnielli VP (2000) Exogenous surfactant kinetics in infant respiratory distress syndrome: a novel method with stable isotopes. Am J Respir Crit Care Med 161(5): 1584–1589. doi:10.1164/ajrccm.161.5.9905088
23. Bartlett GR (1959) Phosphorus assay in column chromatography. J Biol Chem 234(3):466–468
24. Dwyer KP, Barrett PH, Chan D, Foo JI, Watts GF, Croft KD (2002) Oxazolinone derivative of leucine for GC-MS: a sensitive and robust method for stable isotope kinetic studies of lipoproteins. J Lipid Res 43(2):344–349
25. Husek P (1991) Amino acid derivatization and analysis in five minutes. FEBS Lett 280(2):354–356
26. Vedovelli L, Baritussio A, Carnielli VP, Simonato M, Giusti P, Cogo PE (2011) Simultaneous measurement of phosphatidyl glycerol and disaturated-phosphatidylcholine palmitate kinetics from alveolar surfactant. Study in infants with stable isotope tracer, coupled with isotope ratio mass spectrometry. J Mass Spectrom 46(10):986–992. doi:10.1002/jms.1979
27. Nogee LM (2002) Abnormal expression of surfactant protein C and lung disease. Am J Respir Cell Mol Biol 26(6):641–644. doi:10.1165/ajrcmb.26.6.f241
28. Mulugeta S, Beers MF (2006) Surfactant protein C: its unique properties and emerging immunomodulatory role in the lung. Microbes Infect 8(8):2317–2323. doi:10.1016/j.micinf.2006.04.009
29. Kristensen AR, Gsponer J, Foster LJ (2013) Protein synthesis rate is the predominant regulator of protein expression during differentiation. Mol Syst Biol 9:689. doi:10.1038/msb.2013.47
30. Dargaville PA, South M, McDougall PN (1999) Comparison of two methods of diagnostic lung lavage in ventilated infants with lung disease. Am J Respir Crit Care Med 160(3):771–777. doi:10.1164/ajrccm.160.3.9811048
31. Dushianthan A, Goss V, Cusack R, Grocott MP, Postle AD (2014) Phospholipid composition and kinetics in different endobronchial fractions from healthy volunteers. BMC Pulm Med 14:10. doi:10.1186/1471-2466-14-10