Myristate is selectively incorporated into surfactant and decreases dipalmitoylphosphatidylcholine without functional impairment

Christopher J. Pynn,1,2 M. Victoria Picardi,3 Tim Nicholson,4 Dorothee Wistuba,4 Christian F. Poets,1 Erwin Schleicher,2 Jesus Perez-Gil,3 and Wolfgang Bernhard1

Departments of 1Neonatology and 2Internal Medicine IV, Faculty of Medicine, and 4Department of Chemistry, Eberhard-Karls-University, Tübingen, Germany; and 3Department of Bioquimica y Biologia Molecular, Universidad Complutense, Madrid, Spain

Submitted 11 June 2010; accepted in final form 18 August 2010

Pynn CJ, Picardi MV, Nicholson T, Wistuba D, Poets CF, Schleicher E, Perez-Gil J, Bernhard W. Myristate is selectively incorporated into surfactant and decreases dipalmitoylphosphatidylcholine without functional impairment. Am J Physiol Regul Integr Comp Physiol 299: R1306–R1316, 2010. First published September 1, 2010; doi:10.1152/ajpregu.00380.2010.—Lung surfactant nearly exclusively comprises phosphatidylcholines (PC), together with phosphatidylglycerols and surfactant proteins SP-A to SP-D. Dipalmitoyl-PC (PC16:0/16:0), palmitolymyristoyl-PC (PC16:0/14:0), and palmitoylpalmitoleoyl-PC (PC16:0/16:1) together comprise 75–80% of surfactant PC. During alveolarization, which occurs postnatally in the rat, PC16:0/14:0 reversibly increases at the expense of PC16:0/16:0. As lipoproteins modify surfactant metabolism, we postulated an extrapulmonary origin of PC16:0/14:0 enrichment in surfactant. We, therefore, fed rats (d19 –26) with trilaurin (C12:03), trimyristin (C14:03), tripalmitin (C16:03), and trilinolein (C18:23) vs. C14:03, C16:03, C18:13, and C18:23 had no impairment of surface tension function. Combined phospholipase A2 assay and mass spectrometry revealed that 50% of the PC16:0/14:0 peak comprised its isomer 1-myristoyl-2-palmitoyl-PC (PC14:0/16:0). While C12:0 was excluded from incorporation into PC, it increased PC16:0/14:0 as well. C16:0, C18:1, and C18:2 had no significant effect on PC16:0/16:0 or PC16:0/14:0. d3-C14:0 was enriched in lung PC, either via direct supply or via d3-C12:0 elongation. Enrichment of d3-C14:0 in surfactant PC contrasted its rapid turnover in plasma and liver PC, where its elongation product d3-C16:0 surmounted d3-C14:0. In summary, high surfactant PC16:0/14:0 during lung development correlates with C14:0 and C12:0 supply via specific C14:0 enrichment into lung PC. Surfactant that is high in PC16:0/14:0 but low in PC16:0/16:0 is compatible with normal respiration and surfactant function in vitro.

address for reprint requests and other correspondence: W. Bernhard, Dept. of Neonatology, Faculty of Medicine, Eberhard-Karls-Univ., Calwer Straße 7, D-72076 Tübingen, Germany (e-mail: wolfgang.bernhard@med.uni-tuebingen.de).

In vitro, PC16:0/14:0 mimics the effects of natural lipid extract surfactant on the differentiation of macrophages and macrophage-dependent inhibition of T-cell proliferation (17), suggesting potential immunoregulatory functions that may be important for parenchyma protection during development. Additionally, the diagnostic and pathophysiological potential of PC16:0/14:0 has been highlighted by the findings that its concentration in neonatal surfactant correlates better with lung maturity than PC16:0/16:0, and that PC16:0/14:0 may be decreased in chronic lung diseases (7, 9, 21). Such variability is well described for surfactant proteins, which define the presence of tubular myelin, opsonization of pathogens, and surface activity under dynamic conditions, but it is less well understood for the major surfactant phospholipids (6, 36, 38).

In humans and guinea pigs, PC16:0/14:0 increases from alveolarization in utero onward. In the rat and mouse, this increase occurs during postnatal alveolarization (7, 9, 21). In vitro, PC16:0/14:0 mimics the effects of natural lipid extract surfactant on the differentiation of macrophages and macrophage-dependent inhibition of T-cell proliferation (17), suggesting potential immunoregulatory functions that may be important for parenchyma protection during development. Additionally, the diagnostic and pathophysiological potential of PC16:0/14:0 has been highlighted by the findings that its concentration in neonatal surfactant correlates better with lung maturity than PC16:0/16:0, and that PC16:0/14:0 may be decreased in chronic lung diseases (7, 9, 21). Such variability is well described for surfactant proteins, which define the presence of tubular myelin, opsonization of pathogens, and surface activity under dynamic conditions, but it is less well understood for the major surfactant phospholipids (6, 36, 38). Most analyses of surfactant report high concentrations of PC16:0/16:0 as its principal surface tension-lowering compound (23, 52). However, recent detailed lipidomic analyses have also revealed important contributions from PC16:0/14:0 and PC16:0/16:1. These components are specifically enriched in the alveolar space, comprising up to 15 and 28%, respectively, of mammalian surfactant and are fluidic at 37°C in contrast to PC16:0/16:0 (7, 9, 25). This is in stark contrast to the rigid avian lung, where surfactant nearly exclusively comprises PC16:0/16:0 and SP-B (6).

In humans and guinea pigs, PC16:0/14:0 increases from alveolarization in utero onward. In the rat and mouse, this increase occurs during postnatal alveolarization (7, 9, 21). In vitro, PC16:0/14:0 mimics the effects of natural lipid extract surfactant on the differentiation of macrophages and macrophage-dependent inhibition of T-cell proliferation (17), suggesting potential immunoregulatory functions that may be important for parenchyma protection during development. Additionally, the diagnostic and pathophysiological potential of PC16:0/14:0 has been highlighted by the findings that its concentration in neonatal surfactant correlates better with lung maturity than PC16:0/16:0, and that PC16:0/14:0 may be decreased in chronic lung diseases (7, 9). PC16:0/16:0, PC16:0/14:0, and PC16:0/16:1 are selectively enriched in surfactant compared with other lung tissue PC (7). Together, these components comprise 75–80% of surfactant PC (7, 9). This is due to the selective nature of PC accumulation in the lamellar bodies of mammalian type II pneumocytes (PN-II), where selection occurs according to fatty acyl chain length, possibly involving the ABC-A3 transporter system of the lamellar body outer membrane (14, 27, 31). When comparing the PC species composition across vertebrates or during development in vivo, PC16:0/14:0 and PC16:0/16:0 are regulated in an antiparallel fashion (7). By contrast, hormonal stimulation of lung maturation and surfactant synthesis in vivo or in isolated PN-II increases PC16:0/16:0 and PC16:0/14:0 synthesis and pools in a parallel fashion (11, 19, 35). Furthermore, the pattern of newly synthesized PC in isolated PN-II or lung explants is defined by the surrounding medium rather than by hormonal stimulation or lung maturation (11, 12). As lipids from plasma lipoproteins have been shown to stimulate surfactant formation in rats (42), developmental changes in surfactant PC composition may depend on exogenous lipid supply to the lungs.
There are several factors defining the ability of PN-II to produce surfactant. First, PN-II have the ability to acquire substrates such as choline, phosphate, and glucose from the circulation. Second, PN-II are capable of synthesizing fatty acids and glycerides from glucose and stored glycogen (40). Third, PN-II are supplied with fatty acids from triglycerides stored in lipid interstitial cells of the lungs, as well as from lipoproteins and free fatty acids from the circulation (30, 42, 51). After birth, when rat pups regularly feed on milk, lipid interstitial cells and their triglyceride granules increase by two-fold (43). Also PC16:0/14:0 concentration in surfactant is increased at the expense of PC16:0/16:0, while shortly after switching to a carbohydrate-based chow diet, PC16:0/14:0 drops and PC16:0/16:0 increases toward adult values (38). Since rat milk is rich in fatty acids containing less than 16 carbon units, namely myristic (C14:0) and lauric (C12:0) acid, the surfactant PC molecular profile.

We, therefore, fed rats from d19 to d26 on defined synthetic triglycerides, comprising either lauric (C12:0), myristic (C14:0), palmitic (C16:0), oleic (C18:1), or linoleic (C18:2) acid vs. a carbohydrate-based control. We compared changes in the surfactant PC profile with triglyceride and fatty acid composition in lung tissue and plasma, using HPLC and gas chromatography. The effects of C12:0 and C14:0 contrasted those of the other fatty acids and the carbohydrate-based control. Deuterated lauric (ω-d3-C12:0) and myristic (ω-d3-C14:0) acid were used to address the specificity of C14:0 enrichment in surfactant PC, while hydrolysis with phospholipase A2 together with electrospray ionization tandem mass spectrometry (ESI-MS/MS) of the resulting lyso-PC was used to discriminate PC16:0/14:0 from its isomer myristoyl-palmitoyl-PC (PC14:0/16:0). Our data suggest that C14:0 enrichment in surfactant PC is regulated by its supply to the lungs, either directly or via elongation of C12:0. High C14:0 supply induced the formation of dimyristoyl-PC (PC14:0/14:0) and increased that of PC16:0/14:0 and its isomer myristoyl-palmitoyl-PC (PC14:0/16:0). The C14:0-induced biochemical inversion from PC14:0/16:0 to PC16:0/16:0-deprived surfactant did not impair animal growth, respiration, or in vitro surfactant function.

MATERIALS AND METHODS

Materials. Phospholipid standards were from Sigma-Aldrich (Deisenhofen, Germany) or Avanti Polar Lipids (Alabaster, AL), while fatty acid standards were from CDN Isotopes (Pointe Claire, Quebec, Canada), Hydrogen peroxide (30%, analytical grade) was from Boehringer Ingelheim (Ingelheim, Germany), whereas perchloric acid (70%, analytical grade) was from Merck (Darmstadt, Germany). Cholic acid and pancreas phospholipase A2 were from Sigma. Chloroform and methanol were of HPLC grade and from Baker (Deventer, The Netherlands). All other chemicals (analytical grade) were obtained from various commercial sources.

Animal maintenance and feeding experiments. Sprague-Dawley rats of either sex were kept under specific pathogen-free and germ-free conditions and had ad libitum access to an irradiated (5 Mrad) carbohydrate-rich standard rodent diet (Provimi Klibia SA 3336 containing 5.5% fat) and autoclaved water (134°C, 50 min). All animals were spontaneously delivered (term: 21–23 days after conception). For feeding experiments, animals were separated from their mothers at day 19 and put on their respective diets. D19 was chosen to ensure that the animals were mature enough to feed themselves. Experiments were approved by the local governmental authorities and met the National Institutes of Health’s Guidelines for the Care and Use of Laboratory Animals.

Nutrition with synthetic triglycerides. To compare the effects of individual fatty acids, rats were fed ad libitum from day 19 until death at day 26 on a diet based on 20% (wt/vol) of an experimental food for rat and mouse, according to the American Institute of Nutrition (37) (EF R/M acc. AIN 93G water soluble, Sniff Spezialdiäten, Soest, Germany) (EF R/M) ad libitum. Dry EF R/M powder was dissolved in 60°C tap water, cooled to room temperature, and stored at −20°C until use. This control diet supplied 61% and 24% of energy as carbohydrate and protein, respectively, and contained no C12:0, 0.03% C14:0, 0.76% C16:0, 1.86% C18:1, and 3.74% C18:2 (wt/wt). To assess the effects of individual fatty acids, the control diet was supplemented with 2% (wt/vol) trilaurin (C12:0), trimyristin (C14:0), tripalmitin (C16:0), triolein (C18:1), or trilinolein (C18:2) as follows. An aliquot of the respective triglycerides, together with 25% wt/wt egg lecithin was dissolved in 5–10 ml chloroform:methanol (2:1) in a glass beaker. The material was distributed on the walls of the beaker, and the solvent was completely evaporated at 37°C under a stream of nitrogen. The beaker was then filled with 98 ml 20% EF R/M per 2 g triglyceride, stirred at 90°C for 1 h, and repeatedly sonicated using a UW2070 sonicator (Bandelin, Berlin, Germany). Care was taken that no lipid material remained in the glass beaker. Suspensions were then aliquoted and stored at −26°C until use. The resulting fatty acid compositions for the respective feeds are given in Table 1. Formulations were supplied in drink bottles ad libitum, and food uptake and growth were monitored twice daily until death.

Labeling with deuterated C12:0 and C14:0. The metabolism of exogenous C12:0 and C14:0 was assessed at d24–26, i.e., from 3–5 days after weaning from milk feeding to carbohydrate-based rat chow. We dissolved 2.44 mmol dodecanoic-12,12,12-d3 acid (d12-C12:0) (500 mg; mol wt = 203.34) or tetradecanoic-14,14,14-d3 acid (d14-C14:0) (563 mg; mol wt = 231.39) together with 125 mg egg lecithin in 5 ml chloroform:methanol (2:1) in a glass beaker, evaporated the solvent under nitrogen and resuspended, yielding a 2% suspension with EF R/M as described above (25 ml). Suspensions were then aliquoted and stored at −26°C. Day 24 rats (54.4 ± 1.2 g body wt) were supplied orally with a bolus dose of 4.92 µmol/g body wt (50 Data are given in g fatty acid/100 ml. Data were calculated from the fatty acid concentrations of 20% (wt/vol) dissolved diet (sniff EF R/M water soluble), as indicated in the manufacturer’s notes, and the amount of the respective fatty acids of the respective triglycerides added (2%, wt/vol). C12:0, lauric; C14:0, myristic; C16:0, palmitic; C18:1-N9, oleic; C16:2-N9, linoleic acid. Bolded values indicate the fatty acid added as triglyceride in the respective diet.

Table 1. Fatty acid supply in the different feeding groups

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control</th>
<th>Trilaurin</th>
<th>Trimyristin</th>
<th>Tripalmitin</th>
<th>Triolein</th>
<th>Trilinolein</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>0.00</td>
<td>1.87</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.01</td>
<td>0.01</td>
<td>1.89</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>C16:0</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>2.05</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>C18:1</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
<td>2.28</td>
<td>0.37</td>
</tr>
<tr>
<td>C18:2</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>2.66</td>
</tr>
</tbody>
</table>

Downloaded from http://ajpregu.physiology.org/ by 10.220.33.1 on 2017-06-29
μg (body wt) d3-C12:0 (n = 6) or d3-C14:0 (n = 8) label using a disposable syringe and were killed 24 h or 48 h after feeding.

Harvesting of blood plasma, bronchoalveolar lavage fluid (BALF) and tissue samples. Rats were killed by intraperitoneal injection, using 100 mg ketamine (Ketamin 10%, selectavet; Dr. Fischer GmbH, Weyarn-Holzolling, Germany) and 20 mg xylazine (Sedazylan; WDT, Garbsen, Germany) per kg body wt, and subsequently bled by puncturing the right ventricle of the beating heart. Blood (0.2–2.7 ml depending on age) was collected into containers containing EDTA against coagulation, and the lungs were perfused with ice-cold isotonic saline via the right ventricle (7) Blood was centrifuged at 1000 g for 10 min to spin down the cells and blood plasma harvested. BALF was harvested by repeatedly lavaging the lungs through a tracheal catheter with ice-cold isotonic saline (4 to 32 ml depending on age). Recoveries were 85 to 90% as previously described (9). BALF samples containing visible signs of blood contamination were discarded. Cells were removed from BALF by centrifugation at 2000 g for 10 min at 4°C. Lungs and livers were excised, snap frozen in liquid nitrogen, and applied to 100 mg Strata NH2 disposable cartridges (Phenomenex, Torrance, CA) or 1,000 nmol phospholipid internal standard and once eluted with another 2 ml of chloroforom. Lipid extraction. Lipids were extracted from BALF and blood plasma, according to Bligh and Dyer (5), while lung and liver tissues were extracted according to Folch et al. (15) and stored at –32°C until analysis. Total phospholipid was quantified by phospholipid phosphorus determination as described by Bartlett (4) after digestion of the organic components at 190°C in the presence of 500 μl 70% perchloric acid (wt/vol) and 200 μl 30% hydrogen peroxide (wt/vol). Triglycerides, free fatty acids, and phosphatidylcholine (PC) molecular species were isolated from the total lipid extracts as described below.

Analysis of PC molecular species, triglycerides, and free fatty acids. PC and triglycerides were isolated from total lipid extracts as described before (34, 36). Briefly, lipid extracts equivalent to 500 μg (plasma, BALF) or 1,000 (lung and liver tissue) nmol phospholipid were dried under a stream of nitrogen, dissolved in 1 ml chloroforom and applied to 100 mg Strata NH2 disposable cartridges (Phenomenex, Torrance, CA). The chloroforom solvent was collected and triglycerides were eluted with another 2 ml chloroforom. The eluate was then adjusted to 4 ml with chloroforom:methanol (2:1), and 2 ml was transferred to a glass vial for further fatty acid analysis by GC or GC-MS (see below). PC species were subsequently eluted with 1 ml chloroforom:methanol (2:1, vol/vol) and collected for HPLC analysis. Free fatty acids were prepared with TLC, according to Stefan et al. (48). For this, lipid extracts (equivalent to 0.5 to 1.5 μmol phospholipid) were applied to 20 x 20 cm silica TLC plates and developed in hexan:diethylether:glacial acetic acid (160:40:6, vol/vol) over 50–60 min using human plasma extract as a standard.

Analysis of PC molecular species. Eluted PC was dried down under a stream of nitrogen and dissolved in 125 μl trifluoroethanol. Analysis was carried out twice for each sample, once with 100 nm dimyristoyl-PC (PC14:0/14:0)/μmol phospholipid internal standard and once without the standard. This was necessary because C14:0-nutrition resulted in the formation of significant amounts of PC14:0/14:0 in the lungs (see RESULTS). HPLC separation of individual molecular species was performed with a 4.6 x 250 mm Sphere Iimage ODS II column (Scharheck, Bad Godesberg, Germany) and postcolumn fluorescence derivative formation in the presence of 1,6-diphenylyl-1,5-hexatriene as described before (34). To differentiate between PC16:0/14:0 and PC14:0/16:0, which cannot be separated by HPLC due to identical retention times (not shown), PC16:0/14:0 was added to the sample and was separated from HPLC effluents and extracted (5); then, the lipids were hydrolyzed with porcine pancreas phospholipase A2 with minor variations, as described elsewhere (32). Briefly, to 50–100 nmol eluted PC16:0/14:0 + PC14:0/16:0 50–100 nmol cholic acid (2 mmol/l in chloroforom:methanol 2:1, vol/vol) was added, and the solvent was evaporated. Nine-hundred microliters (10 mm) Tris-HCl in 0.9% saline with 10 mM CaCl2, at pH 8.1, and 8–10-2 mm glass beads were added, and the sample was resuspended by vortexing. After adding 100 μl phospholipase A2 (in Tris-HCl, 500 U/ml), the sample was incubated at 37°C in a shaking water bath, and the resulting lyso-PC measured via mass spectrometry with minor modifications as described elsewhere (8). Eicosanoyl-lyso-PC (lysoPC20:0) was used as an internal standard (1 nmol/ml), while electrospray ionization tandem mass spectrometry (ESI-MS/MS) of lysoPC species was performed on a TSQ Quantum Discovery Max triple quadrupole mass spectrometer (Thermo Scientific, Dreieich, Germany) equipped with an electrospray ionization interface. Samples were dissolved in butanol:methanol:water (75:23:2, vol/vol) at a concentration of 1 μmol/l and introduced into the mass spectrometer by loop injection (25 μl) using a Finnigan Surveyor Autosamples plus and eluted with butanol:methanol:water:25% ammonia (75:23:1:7.03; vol/vol) as the mobile phase (0–2 min: 30 μl/min, then 30 to 200 ml/min from 2–3.99 min; back to 30 μl/min at 4 min) using a Finnigan Surveyor MS pump plus (Thermo Scientific). Following fragmentation with argon gas, a fragment with m/z = +184, corresponding to the protonated phosphatidylcholine headgroup, was used for precursor scans of the samples from masses 400–900 and in the selective reaction monitoring mode for individual PC and lysoPC species. This allowed for the assessment of gas purity of the collected subscription PC16:0/14:0 + PC14:0/16:0 peak (m/z = 706) and its complete hydrolysis to form lysoPC14:0 (m/z=468) and lysoPC16:0 (m/z = 496) within 1 h. Values were corrected for C13 isotope effects due to increasing carbon content as described elsewhere (8).

Analysis of endogenous fatty acids from free fatty acids, triglycerides, and PC. Analysis of individual free fatty acids and fatty acids from triglycerides and PC was performed with gas chromatography after (trans)methylation using 13,16,19-docosatrienoic (C22:3) as an internal standard (26). In brief, lipids were dried down at room temperature under a stream of nitrogen. Two milliliters of methanol:toluol (1:3, vol/vol) containing 10 μg/ml C22:3 and 200 μl acetylated cholesterol (99% purity; Fluka, Steinheim, Germany) were subsequently added. Methylation of fatty acids was then performed at 105°C for 60 min in closed Pyrex tubes using a ReactiTherm, model 18971 ( Pierce, Rockford, IL) after which samples were supplemented with 5 ml 6% potassium carbonate, vortexed, and centrifuged for 5 min at 2800 g. The upper phase was concentrated to 180 μl under a stream of nitrogen and transferred to a microvial; 1 μl was injected for gas chromatographic analysis using a HP7673A autosampler (Hewlett Packard, Houston, TX, USA). Analysis was performed on a model HP 5890 gas chromatograph equipped with a split/splitless-injector (230°C) and flame ionization detector (250°C) with hydrogen and synthetic air as combustion gases. A 60 m × 0.25 mm i.d. Rtx 2330 column (Restek, Bellefonte, PA) was used with helium as a carrier gas. Settings for the column were started at 80°C for 2 min, followed by linear increases at a rate of 30°C/min up to 120°C, then 2°C/min increase until a final temperature of 240°C for 10 min. These settings allowed reproducible separation of fatty acids from lauric (C12:0) to long-chain saturated and unsaturated (docosac [C20:0], docosahexaenoic [22:6]) acid in all samples. Quantification was performed using calibration curves for the respective fatty acids.

Analysis of deuterated fatty acid incorporation. Deuterated fatty acid methyl esters, prepared from free fatty acids, triglycerides, and PC (see above), were analyzed via gas chromatography coupled with mass spectrometry (GC-MS) on an Agilent Technologies (Waldbrown, Germany) gas chromatograph (HP6890) equipped with a mass selective detector (HP5973) with electron impact (EI) ionization chamber, a split/splitless injection port, and an autosampler (HP7683). Helium (99.999%) was used as a carrier gas. Separation of analytes was performed on a DB23 capillary column (25 m × 0.25 mm, film thickness 0.15 μm) from J + W Scientific (Folsom, CA). The oven temperature program was as follows: 80°C held for 2 min, ramped to 250°C at 4°C/min and held for 4 min. The injection was made in splitless mode and the injector temperature was 250°C. The ion source temperature and the transfer line were 230°C and 280°C, respectively. The MS system was routinely set in selective ion monitoring mode.
with a solvent delay of 6 min. Agilent Chemstation was used for data collection and GC-MS control. Methyl esters of fatty acids were quantified at their respective mass/charge (m/z) values: lauric acid (m/z = 214), myristic acid (242), palmitic acid (270), and stearic acid (298). Deuterated (M+3) derivatives of these fatty acids were measured at m/z = 217, 245, 273, and 301, respectively, and corrected for the M+3 values from C13 effects of the respective endogenous fatty acids.

Functional characterization of PC16:0/14:0-enriched surfactant. For functional characterization, surfactant enriched in PC16:0/14:0 (C14:03 feeding) was compared with surfactant of normal composition (PC16:0/16:0 enriched; C18:13 feeding). BALF was first ultracentrifuged at 48,000 g for 1 h at 4°C, and the pellets, consisting of surfactant large aggregates, were suspended in 0.9% saline solution. Interfacial adsorption and compression-expansion dynamics of films formed from these surfactant complexes were assessed using a Captive Bubble Surfactometer, similar to that previously described (45), and operated as described elsewhere (18). Aliquots of 150 –200 nl of surfactant at a phospholipid concentration of 8 –10 mg/ml were deposited at the air-liquid interface of a 50 μl air bubble formed inside the Captive Bubble chamber thermostated at 37°C and filled with 5 mM Tris buffer pH 7, containing 150 mM NaCl and 10% sucrose to support the M3 values from C13 effects of the respective endogenous fatty acids. The bubble was imaged with a video camera (Pulnix TM 7 CN) and recorded for later analysis. A 5-min adsorption (film formation) period followed the introduction of the surfactant model suspensions into the chamber during which the bubble was not manipulated and the change in surface tension was monitored (initial adsorption). Afterward, the chamber was sealed and the film-coated bubble rapidly expanded to a volume of 150 μl and imaged for another 5 min to determine postexpansion adsorption. The process was repeated to generate 4 quasi-static compression/expansion cycles, consisting of stepwise reductions in chamber volume to compress the film to the minimum surface tension attainable before the film collapses, followed by successive stepwise increases back to the initial bubble volume. Between each compression and expansion step, there was a 4-s delay, allowing the film to equilibrate, and a 1-min delay between cycles. After the last quasi-static cycle, dynamic cycling was performed, in which the bubble was continuously compressed and expanded over the same volume range determined during the preceding quasi-static cycles, at a rate of 20 cycles/min. For all imaged bubbles, volume, interfacial area and surface tension were calculated from the height and diameter of the bubble, as previously described (44).

Statistical data. were expressed as means ± SE. Three to seven separate experiments were performed for the triglyceride feeding and the respective time points (24 h, 48 h) for labeling with deuterated fatty acids that were all included into statistical analysis. Group differences were tested by a two-tailed Student’s t-test, or by one-way ANOVA for multiple group comparisons using GraphPad Instat ver. 3 (GraphPad Software, San Diego, CA). Multiple group comparisons were corrected using the method of Bonferroni-Holm. P values of <0.05 were regarded as significant.

RESULTS

Effects of exogenous triglycerides on overall growth and neutral lipid parameters. Overall effects of 2% trilaurin (C12:03), trimyristin (C14:03), tripalmitin (C16:03), triolein (C18:13), or trilinolein (C18:23) (for total supply, see Table 1) on growth and neutral and phospholipid parameters are summarized in Table 2. Weight gain in the C18:13 was decreased compared with other groups. C12:03 and C14:03 slightly increased the amount of surfactant phospholipid in BALF, while concentrations of total phospholipid and triglyceride fatty acids were not different compared with controls. The molecular fatty acid profiles of plasma and lung tissue triglycerides are shown in Table 3. Control plasma triglycerides were dominated by C18:2 (34 ± 3%); C18:1 (27 ± 1%); C16:0 (16 ± 2%); and C20:4 (15 ± 2%), while lung tissue triglyceride contained more C16:0 (38 ± 1) and C18:1 (33 ± 1%), but less than 5% C18:2 and C20:4. Also, lung triglyceride contained more C12:0 (3.1 ± 0.3 vs. 0.4 ± 0.1%, P < 0.001), C14:0 (and 5.4 ± 0.2 vs. 0.9 ± 0.2%, P < 0.001) and C16:1 (4.6 ± 0.2% vs. 2.2 ± 0.3%, P < 0.001). Other long-chain (C20:0, C22:0) or polyunsaturated (C20:5, C22:6) fatty acids comprised ~10% in lung tissue triglyceride and less than 5% in plasma triglycerides (not shown).

Exogenous triglycerides increased the respective fatty acid concentrations in both plasma and lung tissue triglycerides (Table 3), except for C18:13 treatment in plasma. Here, the C18:1 fraction was not significantly increased over control values, whereas C18:1 was decreased by all other exogenous triglyceride fatty acids compared with carbohydrate controls. The increases in C12:0 and C14:0 by their respective exogenous triglycerides were much higher than for other fatty acids. In particular, C12:0 and C14:0 were increased in plasma 47- and 22-fold compared with 1.5–1.8-fold for other fatty acids, while in lung tissue, the increases in C12:0 and C14:0 were...
5.3- and 3.5-fold vs. 1.2–2.4-fold for other fatty acids after the respective feedings. Importantly, exogenous C12:0 increased C14:0 in both plasma and lung tissue triglycerides by 2.3 ± 0.4- and 1.5 ± 0.1-fold, respectively, compared with controls (P < 0.001).

Specific effects of exogenous triglycerides on surfactant and lung tissue PC molecular composition. In control rats, surfactant PC comprised 47 ± 1% PC16:0/16:0, 12 ± 1% PC16:0/14:0, and 23 ± 1% PC16:0/16:1. All other components, palmitoyl-oleoyl-PC (PC16:0/18:1), palmitoyl-linoleoyl-PC (PC16:0/18:2), and palmitoyl-arachidonoyl-PC (PC16:0/20:4) were in the range of 4–7% (Fig. 1A). Similarly, lung tissue PC was enriched in these three components, comprising 32 ± 1% PC16:0/16:0, 5.5 ± 0.6% PC16:0/14:0, and 9.2 ± 0.6% PC16:0/16:1. Additionally, lung tissue PC was enriched in PC16:0/18:1 (17 ± 1%), PC16:0/18:2 (10 ± 0.4%), and PC16:0/20:4 (7.2 ± 0.1%), together with minor PC species containing stearic (C18:0) acid like stearoyl-linoleoyl-PC (PD18:0/18:2) and stearoyl-arachidonoyl-PC (PC18:0/20:4) (2–3% each) (Fig. 1B).

Table 3. Composition of the major triglyceride fatty acids in lavaged lung tissue and blood plasma following C12:0-C18:2 triglyceride-rich diets

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control</th>
<th>Trilaurin</th>
<th>Trimyristin</th>
<th>Tripalmitin</th>
<th>Triolein</th>
<th>Trilinolein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C12:0</td>
<td>3.06 ± 0.29</td>
<td>16.10 ± 0.83***</td>
<td>4.30 ± 1.01</td>
<td>1.50 ± 0.17</td>
<td>6.50 ± 0.32</td>
<td>2.06 ± 0.34</td>
</tr>
<tr>
<td>C14:0</td>
<td>5.45 ± 0.21</td>
<td>8.11 ± 0.62</td>
<td>19.21 ± 0.64***</td>
<td>3.27 ± 0.23</td>
<td>7.77 ± 0.14</td>
<td>4.48 ± 0.37</td>
</tr>
<tr>
<td>C16:0</td>
<td>37.66 ± 0.19</td>
<td>32.88 ± 0.45</td>
<td>31.79 ± 0.38</td>
<td>47.21 ± 1.16***</td>
<td>31.29 ± 0.54</td>
<td>38.28 ± 0.50</td>
</tr>
<tr>
<td>C16:1-N7</td>
<td>4.55 ± 0.24</td>
<td>3.99 ± 0.40</td>
<td>4.16 ± 0.40</td>
<td>4.76 ± 0.53</td>
<td>3.39 ± 0.14</td>
<td>4.95 ± 0.20</td>
</tr>
<tr>
<td>C18:1-N9</td>
<td>32.85 ± 0.08</td>
<td>26.03 ± 0.29†††</td>
<td>27.08 ± 0.68†††</td>
<td>26.80 ± 1.11†††</td>
<td>38.38 ± 1.05***</td>
<td>28.12 ± 0.35†††</td>
</tr>
<tr>
<td>C18:2-N6</td>
<td>2.71 ± 0.16</td>
<td>2.25 ± 0.19</td>
<td>2.57 ± 0.14</td>
<td>2.10 ± 0.19</td>
<td>2.32 ± 0.09</td>
<td>6.39 ± 0.13***</td>
</tr>
<tr>
<td>C20:4-N6</td>
<td>3.70 ± 0.38</td>
<td>2.26 ± 0.49</td>
<td>2.56 ± 0.38</td>
<td>3.73 ± 0.56</td>
<td>2.31 ± 0.29</td>
<td>4.81 ± 0.27</td>
</tr>
<tr>
<td>Blood plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C12:0</td>
<td>0.35 ± 0.08</td>
<td>16.56 ± 2.72***</td>
<td>0.31 ± 0.03</td>
<td>0.19 ± 0.02</td>
<td>0.46 ± 0.06</td>
<td>0.19 ± 0.04</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.90 ± 0.20</td>
<td>2.04 ± 0.33</td>
<td>20.14 ± 1.44***</td>
<td>0.87 ± 0.09</td>
<td>0.88 ± 0.04</td>
<td>0.55 ± 0.08</td>
</tr>
<tr>
<td>C16:0</td>
<td>16.27 ± 1.89</td>
<td>13.03 ± 0.83</td>
<td>13.37 ± 0.81†††</td>
<td>29.49 ± 2.66***</td>
<td>12.16 ± 0.59</td>
<td>9.87 ± 0.47</td>
</tr>
<tr>
<td>C16:1-N7</td>
<td>2.23 ± 0.25</td>
<td>1.46 ± 0.22</td>
<td>1.49 ± 0.23</td>
<td>2.32 ± 0.19</td>
<td>0.80 ± 0.10†††</td>
<td>0.83 ± 0.10†+§§</td>
</tr>
<tr>
<td>C18:1-N9</td>
<td>27.31 ± 0.94</td>
<td>15.38 ± 0.63†††</td>
<td>16.17 ± 0.66†††</td>
<td>20.32 ± 0.87†††</td>
<td>30.40 ± 1.34†††</td>
<td>12.28 ± 1.12†††</td>
</tr>
<tr>
<td>C18:2-N6</td>
<td>34.50 ± 3.32</td>
<td>27.27 ± 1.03</td>
<td>25.95 ± 0.73</td>
<td>24.78 ± 1.53</td>
<td>24.18 ± 0.57</td>
<td>52.93 ± 2.56***</td>
</tr>
<tr>
<td>C20:4-N6</td>
<td>14.52 ± 1.61</td>
<td>20.47 ± 1.78</td>
<td>18.81 ± 1.80</td>
<td>17.31 ± 2.09</td>
<td>27.20 ± 1.16†††</td>
<td>20.64 ± 3.37</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE of 4 to 7 experiments. Rats were fed for 6 days on diets containing either no TAG supplementation (control) or with 2% trilaurin, trimyristin, tripalmitin, triolein, or trilinolein. Fatty acids of triglycerides were determined by gas chromatography as described in MATERIALS AND METHODS. C20:4-N6, arachidonic acid.***P < 0.001 vs. all other groups; ††P < 0.01, †††P < 0.001 vs. control; ††††P < 0.001 vs. C12:0, C14:0, C16:0 and C18:2-N6; §§P < 0.01 vs. C16:0.
In surfactant (BALF) and lung tissue, C14:0 increased PC16:0/14:0 by three-fold at the expense of PC16:0/16:0 and PC16:0/16:1, while only in lung tissue, PC16:0/18:1 was decreased (Fig. 1, A and B). Additionally, C14:0 induced the formation of PC14:0/14:0 (7.0 ± 0.3%), which in controls was virtually absent. C12:0 also increased PC16:0/14:0 from 11.6 ± 1.05 to 15.1 ± 0.28% (P < 0.05) and PC14:0/14:0 from 0.14 ± 0.01 to 0.62 ± 0.02% (P < 0.05). C16:0 and C18:1 exerted no significant effects on surfactant PC16:0/14:0, PC16:0/16:0, or PC16:0/16:1 distribution, while C18:2 increased PC16:0/18:2 from 6.5 ± 0.7% to 13.0 ± 0.5%, which was at the expense of PC16:0/16:1 (P < 0.05) and with a small increase in PC16:0/16:0 (55 ± 2% compared with 47 ± 1% in controls; P < 0.05) (Fig. 1A). Similar changes were induced by exogenous triglycerides in lung tissue PC. Additionally, PC18:0/18:2 was increased here by C18:2 (Fig. 1B).

Consistent with these findings, analysis of the fatty acid composition of PC revealed that exogenous C12:0 and C14:0 increased C14:0 in both lung tissue (Table 4) and BALF (not shown). Importantly, while the relative increases in C12:0 and C14:0 in lung tissue PC were in the same range (8.4- and 5.8-fold by the respective diets) as in its triglycerides (Table 3), total incorporation of C12:0 remained low so that in lung tissue PC, it was always below 1% and did not impact on other PC fatty acids, while C14:0 increased up to 19.2 ± 0.6% and decreased C16:0, C16:1, and C18:1 in lung PC (Table 4).

ESI-MS/MS analysis of the PC16:0/14:0 HPLC peaks collected from different samples revealed a mass by charge (m/z) = +706 as their single component, which is equivalent to the masses of both PC16:0/14:0 and PC14:0/16:0, with m/z = +184 (phosphocholine) as a diagnostic fragment (not shown). Complete hydrolysis of this peak with phospholipase A2 generated 1-myristoyl-glycero-3-phosphocholine (lysoPC14:0; m/z = +468) and 1-palmitoyl-glycero-3-phosphocholine (lysoPC16:0; m/z = +496) as the only reaction products, indicating that the PC16:0/14:0 peak from HPLC the analysis was a mixture of 1-palmitoyl-2-myristoyl-PC (PC16:0/14:0) and 1-myristoyl-2-palmitoyl-PC (PC14:0/16:0). In C14:0-fed animals, 44.4 ± 2.0% of the m/z = +706 peak was PC14:0/16:0. This was not different from PC16:0/16:0-enriched surfactant after triolein feeding (46.6 ± 0.9% PC14:0/16:0) or later development (adult, n = 5: 41.7 ± 0.8%) (P > 0.05).

Effects of PC composition on respiratory and surfactant function. Resting respiratory rate of the animals with increased PC16:0/14:0, PC14:0/16:0, and PC14:0/14:0 after C14:0 feeding was not different from PC16:0/16:0-rich animals (126 ± 5 vs. 115 ± 5 breaths/min for C18:1 controls; n = 6; P > 0.05), nor did the animals with such an “inverted” low PC16:0/16:0 surfactant show cyanosis or decreased growth (Table 2). Moreover, in vitro function of inverted surfactant was not different from high-PC16:0/16:0 surfactant, neither initially nor after expansion (Fig. 2A). Both surfactants reached equilibrium surface tensions of 22–24 mN/m within 15 s. Also, minimal surface tension values of below 5 mN/m were readily achieved by both surfactants (Fig. 2B). Under quasi-static conditions, inverted surfactant induced by trimyristin (C14:0; Fig. 2B, bottom left) reached near-zero surface tension during the first compression-expansion cycle with slightly less compression (35% vs. 40%) than normal high PC16:0/16:0 surfactant after triolein feeding (C18:1; Fig. 2B, top left). Under dynamic conditions repeated compression-decompression resulted in nearly identical surface tension behavior of inverted and normal surfactant (Fig. 2B, right).

Metabolism of exogenous d3-C14:0 and d3-C12:0 in lung, plasma, and liver. Of the 4.92 μmol/g body wt d3-C14:0 applied to the rats, 31.4 ± 5.7 nmol and 233 ± 76 nmol were detected in PC from BALF and lavaged lung tissue after 24 h. These values were maintained for 48 h in BALF and lung tissue PC with no significant decreases (Fig. 3, A and B). Whereas the amounts of deuterated palmitic acid (d3-C16:0) tended to increase continuously, deuterated stearic acid (d3-C18:0) was below the limit of detection (Fig. 3, A and B). By contrast, in plasma and liver, PC concentrations of d3-C16:0 surmounted those of d3-C14:0, and d3-C18:0 was present in measurable amounts (Fig. 3, C and D). Moreover, d3-C16:0 and d3-C14:0 in plasma and liver PC peaked at 24 h and then rapidly decreased in contrast to BALF and lung tissue PC (Fig. 3, C and D). Regarding the fatty acid substrates for lung PC synthesis, d3-C14:0 surmounted d3-C16:0 in both lung and plasma free fatty acids and triglycerides, while d3-C18:0 was below the detection level during the 48-h period of investigation (Fig. 4, A–D). Again, for lung free fatty acids and triglycerides d3-C14:0 and d3-C16:0, concentrations were maintained for at least 48 h (Fig. 4, A and C), while in plasma, their concentrations peaked at 24 h and were nearly absent at 48 h (Fig. 4, B and D).

In light of the virtual absence of C12:0 from surfactant PC, but its effect on increasing myristic acid of PC (Table 4, Fig. 1), we investigated the metabolism of its deuterated analog (d3-C12:0) and elongation products (d3-C14:0 and d3-C16:0) in lungs and plasma. After ingestion of 4.92 μmol/g body wt d3-C12:0, this deuterated fatty acid was below detection level in PC of BALF. In contrast, however, lung tissue PC contained...
450 ± 149 pmol/g body wt after 24 h, although by 48 h values were below the detection limit (not shown). By contrast, d3-C14:0, as well as d3-C16:0, increased in lung tissue and surfactant PC (Fig. 5A). Interestingly and in contrast to the d3-C14:0 experiments, absolute values of d3-C16:0 were higher than those of d3-C14:0 (Fig. 5B). d3-C12:0 and its elongation products d3-C14:0 and d3-C16:0, were detected in all potential fatty acid sources for lung PC synthesis, free fatty acids and triglycerides from both plasma and lung tissue (Fig. 5C and D). Significantly, concentrations of d3-C14:0 and d3-C16:0 in lung tissue free fatty acids were much greater than those of d3-C12:0 (Fig. 5C), while in lung tissue triglycerides, as well as in plasma free fatty acids and triglycerides, d3-C12:0 values were much higher than those of d3-C14:0 and d3-C16:0. d3-C12:0 in lung triglycerides peaked at 24 h and then decreased, while its elongation products further increased (Fig. 5D) in contrast to exogenous d3-C14:0 experiments, where the d3-C14:0 concentration was maintained for 48 h in lung tissue triglycerides (Fig. 4C).

**DISCUSSION**

Phosphatidylcholine (PC) synthesis in type II pneumocytes (PN-II) depends on exogenous choline for the formation of the head group, while the glycerol moiety originates from exogenous glucose or endogenous glycogen stores (24, 46, 49). The lung contains all of the enzymes to synthesize, desaturate or elongate fatty acids (for review, see Ref. 39), suggesting that PN-II may possess considerable “autarchy” for generating a PC molecular profile characteristic for surfactant. In utero, the C2-units for palmitic acid (C16:0) synthesis are derived from exogenous glucose or from pulmonary glycogen (40). Such fatty acid synthesis of the lung is essential during fetal development, where 80% thereof are found in PC and other lung phospholipids (10). In most vertebrates surfactant is highly enriched in dipalmitoyl-PC (PC16:0/16:0), the end product of de novo synthesis, as well as acyl remodeling. However, in all mammals myristic acid (C14:0) containing PC species, like PC16:0/14:0 or its isomers and alkyl-acyl-analogs are present,
and sometimes highly enriched, either transiently during alveolar development as in humans, rats, and mice or continuously as in pygmy shrews or some marsupials (7, 9, 25, 38). Although the regulation of surfactant-specific PC components has hitherto been attributed mostly to ontological development and hormonal regulation, our data suggest that exogenous supply of the lungs with saturated fatty acids shorter than C16:0, particularly C14:0, may be a major determinant of surfactant PC composition.

Role of exogenous fatty acid supply to the PN-II for surfactant PC homoeostasis. PN-II utilize exogenous fatty acids, triglycerides and very low-density lipoproteins to synthesize surfactant PC (13, 16, 42), suggesting that neutral lipids and lipoproteins are intimately linked to surfactant metabolism. In this context, the data presented here show that the fatty acid composition of exogenous triglycerides differentially influence the PC profile of lung surfactant. While all fatty acids supplied change the fatty acid composition in plasma and lung tissue triglycerides to similar extents, the effects of C12:0 and C14:0 were most significant compared with their concentrations in carbohydrate controls or rats fed on longer fatty acids. Furthermore, supply with exogenous C14:0 increased the myristoylated PC components of surfactant at the expense of PC16:0/16:0, while exogenous supply with C16:0, C18:1, and C18:2

Fig. 3. Incorporation of deuterated myristic acid (d1-C14:0) and its elongation products palmitic (d1-C16:0) and stearic (d1-C18:0) acid into lung, liver, and plasma PC. Rats were fed 4.92 µmol/g body wt d1-C14:0 and its incorporation and those of its elongation products d1-C16:0 and d1-C18:0 into the PC fractions of surfactant (BALF) (A), lavaged lung tissue (B), blood plasma (C), and liver (D) determined. Deuterated fatty acids were determined by GC-MS as described in MATERIALS AND METHODS. Data are expressed as means ± SE of 4 experiments per data point. A, B: *P < 0.05, ***P < 0.001 vs. d3-C16:0 and d3-C18:0 of corresponding time points; C, D: †††P < 0.001 vs. d3-C14:0 and d3-C18:0 of corresponding time point; ‡‡P < 0.01, ‡‡‡P < 0.001 vs. 24 h of respective fatty acid.

Fig. 4. Incorporation of deuterated myristic acid and its elongation products into free fatty acids and triglycerides of lung tissue and blood plasma. Rats were fed 4.92 µmol/g body wt d1-C14:0 and its presence and those of its elongation products palmitic (d1-C16:0) and stearic (d1-C18:0) acid determined in the free fatty acid (A, B) and triglyceride (C, D) fractions of lung tissue (A, C) and blood plasma (B, D). Deuterated fatty acids were determined after methylation by GC-MS as described in MATERIALS AND METHODS. Data are expressed as means ± SE of 4 experiments per data point. A, D: **P < 0.01, ***P < 0.001 vs. d3-C16:0 and d3-C18:0 of the corresponding time points; B, D: ††P < 0.01, †††P < 0.001 vs. 24 h of the respective fatty acid (d1-C14:0, d1-C16:0).
had no major effect on PC composition, which is consistent with previous findings showing minor effects on PC16:0/16:0 concentrations when mainly feeding fatty acids longer than 16 carbon units, although supply with linoleic or long-chain polyunsaturated fatty acids may exert increased inflammatory reactions of the lung (2, 53). These data are also in line with Longmuir and Rossi (28), showing that the lung does not shorten fatty acids to a significant extent to generate C14:0. Taken together, our data suggest that C14:0 is preferentially enriched in the lungs, and C14:0 supply to the lungs is the most significant exogenous modulator of surfactant PC profile.

In the rat and mouse, PC16:0/14:0 increases postnatally, correlating with the high supply of milk containing C12:0 and C14:0 (47), while such changes occur antenatally in other species, such as humans and guinea pigs (7, 9, 21, 38). These differences imply different mechanisms of regulation across mammalian species. Fatty acids from very low density lipoproteins have been shown to be incorporated into PC (42), and data from other groups (2) exclude a major hormonal control of surfactant PC profile. Hence, we postulate that the fractions of PC16:0/14:0 and PC16:0/14:0 in surfactant are determined by the supply of the lungs with C14:0 from neutral lipids. This metabolic principle may be true for both, direct supply from plasma lipoproteins, as well as triglycerides stored in pulmonary lipid interstitial cells (50, 51).

**Effects of molecular inversion of surfactant PC profile on surface tension function.** The view that mammalian surfactant is highly enriched in PC16:0/16:0, a critical component deemed necessary for lowering alveolar surface tension (1, 22), has recently been challenged since surfactant contains very low PC16:0/16:0 concentrations in several mammals (7, 25). Consistent with this, in vitro observations have shown that the biophysical properties of the overall surfactant phospholipid mixture are markedly different from those of the individual components (33). In this context, it is not surprising that a surfactant with an “inverted composition,” containing only 29% PC16:0/16:0, 45% PC16:0/14:0 and its isomer PC14:0/16:0, as well as even 7% PC14:0/14:0 relative to total PC, shows good surface tension function in vitro, and, judging by the normal development and breathing rates of the C14:03-fed rats, in vivo. PC16:0/14:0 is possibly important for the immune homeostasis of developing lungs due to its effects on macrophages (17). Interestingly, PC16:0/14:0 is not present in surfactant of healthy bird lungs, which only contain interstitial macrophages not exposed to the surfactant-containing air spaces (29). On the other hand, phase transition temperatures of PC14:0/14:0, PC16:0/14:0 and PC14:0/16:0 are below body temperature (transition temperatures: 23°C, 27°C, 35°C, respectively) compared with PC16:0/16:0 (41.5°C) (20). Although this may well decrease the viscosity and possibly
improve therapeutic applicability of surfactant (41), further research will be necessary to evaluate its function in vivo at variable body temperatures. However, since artificial PC16:0/16:0-based surfactants appear to be less effective than natural surfactants, inclusion of physiological surfactant components, such as PC16:0/14:0 should be considered in the construction of future artificial lung surfactants.

Metabolism of C14:0 in the lungs relative to plasma and liver. The preferential enrichment of exogenously labeled d3-C14:0 in lung tissue and surfactant PC is in clear contrast to the liver, where preferentially, its elongation products d3-C16:0 & d3-C18:0 were incorporated into PC and where turnover was much faster. Moreover, while d3-labeled C12:0 was effectively excluded from incorporation, its elongation products, d3-C14:0 & d3-C16:0 were enriched in lung PC, highlighting the specificity of pulmonary fatty acid incorporation into surfactant. This is consistent with the preference of the lungs to incorporate exogenous C14:0, and with data on human volunteers showing a smaller turnover of surfactant compared with plasma PC (8). The results presented here extend those findings to the neutral lipids, since in lung tissue triglycerides and free fatty acids, the accumulation of d3-C14:0 and d3-C16:0 persisted, resembling the overall low lipid turnover of the lungs, while in plasma (see Fig. 5, B and D) and liver (not shown) d3-labeled free fatty acids and triglycerides had declined to near zero values after 48 h.

Another specific indicator of the lung’s selectivity of d3-C14:0 over d3-C12:0 metabolism is their turn over time in the neutral lipid fraction of the lungs compared with plasma. Although in lung and plasma triglycerides, as well as in plasma free fatty acids, d3-C12:0 concentrations far exceeded those of d3-C14:0 (and d3-C16:0), in lung tissue free fatty acids d3-C14:0 as a fatty acid source of PC assembly exceeded d3-C12:0 (Fig. 5, C and D). This rules out that lung tissue free fatty acids are merely an overflow of plasma or that they simply resemble the hydrolysis products of lung tissue triglycerides. Instead, d3-C14:0 (and d3-C16:0) must either be preferentially absorbed from the circulation as free fatty acids or effectively elongated from pulmonary and extrapulmonary triglyceride stores. Hence, C14:0 is specifically enriched in surfactant PC by either direct supply or via elongation of shorter fatty acids (e.g., C12:0).

Perspectives and Significance

The molecular profile of surfactant PC is related to lung physiology, and to the supply and metabolism of neutral lipids, where myristic acid is specifically enriched in the lung, and has the most direct effect on components preferentially sequestered into the alveolar space. A significant contribution not only of PC16:0/16:0, but also of PC16:0/14:0 and PC16:0/16:1 to lung surfactant is characteristic of all mammals, including humans. Respiratory function in vivo and surface tension function in vitro of organisms possessing “inverted” surfactants with high PC16:0/14:0, considerable PC16:0/16:1 and low PC16:0/16:0 suggest that inclusion of all these PC components, rather than only of PC16:0/16:0, should be considered when constructing synthetic therapeutic surfactants. Nevertheless, several issues have yet to be addressed: do the actions of PC16:0/14:0 on macrophage differentiation and function in vitro (17) hold also true for alveolar immune homeostasis in vivo, where PC16:0/14:0 is particularly increased during alveolar formation (7)?

Moreover, are these in vitro actions of surfactant PC16:0/14:0, eventually together with the general accumulation of C14:0 in other lung lipids, important for inflammatory reactions, where unsaturated fatty acid supply appears to be harmful rather than protective to the lung (53)? Finally, it will be essential to address the safety and efficacy of PC16:0/14:0-enriched surfactants in vivo under pathological conditions. These include maintenance of alveolar stability during hypothermia or fever, as frequently found in preterm neonates and older patients in the intensive care setting.

ACKNOWLEDGMENTS

The authors thank Verena Müller, Marco Raith, and Jamila Borchert for excellent technical assistance.

GRANTS

This work was supported by an institutional grant of the medical faculty of the University of Tübingen (F 1275127) and by the German Research Council (BE2223/2-1). V. P. and J. P.-G. were supported by grants from the Spanish Ministry of Science (BIO2009-09694, CSD2007-00010) and the Community of Madrid (P-MAT-000283-0505).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


Downloaded from http://ajpregu.physiology.org/ by 10.220.33.1 on June 29, 2017
MYRISTIC ACID DECREASES SURFACTANT DIPALMITOYLPHOSPHATIDYLCHELONE

R1316

Ikegami M, Jobe A.

18. Gomez-Gil L, Goormaghtigh E, Perez-Gil J.


