Altered fatty acid composition of lung surfactant phospholipids in interstitial lung disease


Altered fatty acid composition of lung surfactant phospholipids in interstitial lung disease. Am J Physiol Lung Cell Mol Physiol 283: L1079–L1085, 2002. First published July 12, 2002; 10.1152/ajplung.00484.2001.—Deterioration of pulmonary surfactant function has been reported in interstitial lung disease; however, the molecular basis is presently unclear. We analyzed fatty acid (FA) profiles of several surfactant phospholipid classes isolated from large-surfactant aggregates of patients with idiopathic pulmonary fibrosis (IPF; n = 12), hypersensitivity pneumonitis (n = 5), and sarcoidosis (n = 12). Eight healthy individuals served as controls. The relative content of palmitic acid in phosphatidylethanolamine was significantly reduced in IPF (66.8 ± 2.5%; means ± SE; P < 0.01) but not in hypersensitivity pneumonitis (78.5 ± 1.8%) and sarcoidosis (78.2 ± 3.1%; control 80.1 ± 0.7%). In addition, the phosphatidylglycerol FA profile was significantly altered in the IPF patients, with a lower relative content of its major FA, oleic acid, at the expense of saturated FA. In the phosphatidylethanolamine class, a significant correlation between the impairment of biophysical surfactant function and decreased percentages of palmitic acid was noted. We conclude that significant alterations in the FA profile of pulmonary surfactant phospholipids occur predominantly in IPF and may contribute to the disturbances of alveolar surface activity in this disease.

pulmonary surfactant; idiopathic pulmonary fibrosis; hypersensitivity pneumonitis; sarcoidosis; surface activity

PULMONARY SURFACTANT IS A complex mixture of lipids and proteins, which reduces the surface tension at the alveolar air-liquid interface to values near 0 mN/m, thereby stabilizing the alveoli and preventing them from collapse. The major phospholipid (PL) component, dipalmitoylphosphatidylcholine (DPPC), is crucial for the extreme reduction of surface tension during expiration, as it may be overcompressed as a monolayer (35) because of its physicochemical properties (critical transition temperature, 41°C) at physiological body temperature. Other surfactant components, such as unsaturated PLs, in particular phosphatidylglycerol (PG), or hydrophobic proteins [surfactant apoprotein (SP)-B and SP-C], are centrally involved in the adsorption and compression behavior of the monolayer (17).

Alterations in the biochemical composition and the biophysical properties of pulmonary surfactant are well documented for patients with acute inflammatory lung diseases such as the acute respiratory distress syndrome (8, 9, 11). In these patients, alterations of both the lipid moiety and the surfactant-specific apoproteins were noted, with a reduced relative content of phosphatidylethanolamine (PC) and PG, an increase in minor PL including sphingomyelin (SPH), phosphatidylinositol (PI), and phosphatidylethanolamine (PE), a decreased relative content of disaturated PC species, increased lavagable protein fraction, and reduced SP-A, SP-B, and SP-C. In contrast, reports on the composition of surfactant from patients with chronic interstitial lung diseases (ILDs) are scarce. Previous studies mainly focused on the overall PL and protein content in bronchoalveolar lavage fluid (BALF), distribution of PL classes, and content of surfactant-specific proteins (2, 15, 19, 20, 21, 29). Concerning the fatty acids (FAs), Honda et al. (15) reported decreased levels of DPPC in patients with ILDs, whereas Baughman et al. (2) found a highly elevated relative content of palmitic acid in PC isolated from BALF of patients with sarcoidosis (Sarc). There are currently no data concerning the FA profile of minor surfactant PLs in ILD.

In the present study, we determined the PL class profile and the FA composition of choline, glycerol, inositol, ethanolamine, serine, and SPH PL of pulmonary surfactant isolated from 8 healthy volunteers and 29 patients with chronic ILDs, including idiopathic pulmonary fibrosis (IPF), hypersensitivity pneumonitis (HP), and Sarc. We found an impressive decrease in palmitic acid residues of the most important PL component, PC, in patients suffering from IPF. A significant correlation between the decrease in the percentage of palmitic acid in PC and the impairment of biophysical surfactant function in IPF patients was...
observed. Changes in the FA composition of surfactant PLs may thus contribute to alveolar collapse and impairment of gas exchange in IPF.

METHODS

Patient Population

This study was conducted at the Department of Internal Medicine, Justus-Liebig-University in Giessen, Germany. It was approved by the local ethics committee, and informed consent was obtained from each individual before entering into the study and subsequent bronchoscopy. The spontaneously breathing patients underwent fiber-optic bronchoscopy for various diagnostic indications. The analysis described here was performed with remaining aliquots of BALF. None of the patients was treated for ILDs at the time of initial lavage. The control group consisted of eight healthy volunteers who had never smoked and had no history of cardiac or pulmonary disease. They also showed normal chest X-rays and pulmonary function tests. There was no overlap between the groups of patients. Demographic and clinical data for the patient groups and controls are summarized in Table 1. Routine tests for lung function were performed in all patients. At the time of the study, patients with HP and IPF were reported to have had complaints for 15–40 mo, paralleled by progressive dyspnea (Table 1). Accordingly, lung function both at rest and during exercise was impaired. Patients with Sarc reported a slightly shorter duration of complaints and displayed less dyspnea and more moderate alterations in lung function (Table 1). All patients with ILD (a total of 29) were classified into IPF, HP, and Sarc, according to the following criteria.

IPF. The patients included in this group (n = 12) were requested to show a slowly progressive lung fibrosis, with an insidious onset of >6 mo. Inspiratory crackles and finger clubbing were present in all patients. All patients displayed a restrictive pattern of lung function, in combination with a reduced CO diffusion capacity. In addition, chest X-rays or high-resolution computed tomography (CT), which were performed in all patients, had to show a bilateral fine or coarse reticular pattern with predominance of the basal and subpleural lung regions. Ground glass opacities had to be absent or scarce, whereas honeycombing was easily detected, preferably in the basal and subpleural regions. Patients were not allowed to be included if they had a nodular pattern, extensive ground glass opacities, or asymmetric distribution, or if they showed hilar or mediastinal adenopathy. Another mandatory criterion for inclusion was proof of an increase in neutrophil counts in the bronchoalveolar lavage, with possible minor and concomitant increase in lymphocyte or eosinophil counts.

In addition to these investigations, considerable effort was undertaken to exclude other diseases that could mimic IPF. In detail, all patients had to fill out a standardized questionnaire (18), which, in addition to other aspects, included 1) a detailed history of all professional and other activities, 2) a detailed history of all preceding medications, 3) a detailed description of comorbidity, and 4) a detailed description of the nature, duration, and possible aggravation of complaints. Absence of collagen or vascular diseases was ascertained by a complete immunological screening and by clinical examination (e.g., no sign of renopulmonary syndrome). Patients included in this group were human immunodeficiency virus negative and were free from hepatitis infection and chronic bowel disease. The exclusion of an alternative diagnosis was also based on the transbronchial lung biopsy, which was performed on all patients. Transbronchial biopsy did not reveal an alternative diagnosis but showed changes in accordance with a usual interstitial pneumonitis pattern in 6 of 12 patients. No alveolar structures were seen in the remaining six patients, one patient of whom required open-lung biopsy, which, indeed, showed a usual interstitial pneumonitis pattern. Because most patients in this study were included before publication of the American Thoracic Society and European Respiratory Society Consensus Conference on IPF, patients’ files were again analyzed on a retrospective basis. All included patients fulfilled the consensus criteria (1).

HP. This group included patients (n = 5) with proof of a hypersensitivity response to an inhalative antigen (history of periodically recurring or permanent complaints on exposure, in combination with the detection of precipitating antibodies). The precipitating antibodies were directed against pigeon feathers (n = 3) and canary feathers (n = 1) in the four patients with avian-induced HP and against thermoactinomyces vulgaris in the one patient with farmer’s lung. Patchy interstitial infiltrations, mostly of nodular character and with a patchy or homogeneous distribution, were found in both lungs in chest X-rays or CT or high-resolution CT. BALF lymphocyte counts were elevated, with a decreased CD4-to-CD8 ratio. Transbronchial biopsies were performed in all patients and revealed typical alterations of HP in three of five patients. These changes included signs of alveolar and interstitial inflammation, with an alveolar exsudate and in-

| Table 1. Demographic and clinical data on initial lavage and cell counts |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Control         | IPF             | HP              | Sarc            |
| Age, yr                        | 44.9 ± 4.6      | 52.0 ± 4.1      | 53.5 ± 5.6      | 38.8 ± 2.7      |
| Gender (female/male)           | 3/5             | 6/6             | 4/1             | 4/8             |
| Smoking (NS/ES/CS)             | 8/0/0           | 7/5/0           | 5/0/0           | 7/5/0           |
| FVC, %predicted                | ND              | 60.0 ± 5.5      | 62.0 ± 4.7      | 78.7 ± 4.3      |
| Duration of complaints, mo     | 33.3 ± 8.6      | 26.7 ± 5.2      | 20.1 ± 6.2      |                 |
| Dyspnea score                  | 3.6 ± 0.3       | 1.4 ± 0.5       | 1.0 ± 0.5       |                 |
| Neutrophils, %                 | 2.89 ± 0.73     | 31.22 ± 7.33*   | 16.23 ± 5.02†   | 3.94 ± 1.26     |
| Lymphocytes, %                 | 6.92 ± 1.36     | 10.92 ± 3.11    | 34.44 ± 5.25‡   | 18.00 ± 2.92*   |
| Macrophages, %                 | 90.19 ± 1.21    | 57.86 ± 7.95†   | 49.33 ± 6.60‡   | 78.06 ± 3.41*   |
| CD4/CD8                       | ND              | 1.09 ± 0.10     | 0.51 ± 0.22     | 3.32 ± 0.66     |

Values are means ± SE; n, no. of subjects. Dysnea was individually scored by each patient in a scale from 0 to 4. Control, healthy volunteers; IPF, idiopathic pulmonary fibrosis; HP, hypersensitivity pneumonitis; Sarc, sarcoidosis; NS, never smoker; ES, ex-smoker; CS, current smoker; FVC, forced vital capacity; CD4/CD8, ratio of CD4+ to CD8+ cells; ND, not determined. *P < 0.05; †P < 0.01; ‡P < 0.001, compared with controls.
creased numbers of lymphocytes, macrophages, and also gi-
ant cells. In the remaining two patients, the biopsies could not be evaluated because of a lack of alveolar tissue. No surgical biopsy was performed in this patient group.

Sarc. These patients (n = 12) presented with abnormal chest radiographs (bilateral hilar adenopathy without (stage I, n = 1) or with (stage II, n = 6) pulmonary infiltrates, such as coarse reticulonodules or fluffy cotton wool confluent shadows, or infiltrates without hilar adenopathy (stage III, n = 5). The BALF lymphocyte counts were elevated with an increased CD4-to-CD8 ratio (in all 12 patients), and typical granulomas were found in lung biopsies of 9 out of 12 patients and in all 5 patients with stage III. In three patients (1 with stage I, 2 with stage II), transbronchial biopsy did not include alveolar tissue and thus could not be used for diagnostic proposes. No patient of this group underwent open-lung biopsy.

**Bronchoalveolar Lavage Technique**

Flexible fiber-optic bronchoscopy was performed in a standardzied manner as previously described (9). One segment of the lingula or the right middle lobe was lavaged with a total volume of 200 ml sterile saline in 10 aliquots. The fluid recovery was found to range at 65.8% volume of 200 ml sterile saline in 10 aliquots. The BALF lymphocyte counts were elevated with an increased CD4-to-CD8 ratio (in all 12 patients), and typical granulomas were found in lung biopsies of 9 out of 12 patients and in all 5 patients with stage III. In three patients (1 with stage I, 2 with stage II), transbronchial biopsy did not include alveolar tissue and thus could not be used for diagnostic proposes. No patient of this group underwent open-lung biopsy.

**Lipid Analysis of BALF**

**PL content and PL profile.** Lipids were extracted from BALF with chloroform-methanol (3), and the PL content was determined by spectrophotometric measurement of phospho-
rus, according to the method of Rouzer et al. (30). Individual PLs were separated by high-performance thin-layer chromatography, as previously described (9), by using silica 60 plates (Merck, Darmstadt, Germany) and chloroform-meth-

anol-acetic acid-water (50:37.5:3:5:2, vol/vol/vol/vol) as de-
volutionary. This one-dimensional system allows the separation of eight PL classes: lyso-PC, SPH, PC, phosphatidylserine (PS), PI, PE, PG, and cardiolipin. Samples (25 μg total PL) and seven concentrations of each PL standard (all from Sigma Chemical) were applied to the plate with a Linomat IV applicator (Camag, Muttenz, Switzerland) and stained with molybdenum blue reagent, according to the method of Gustavsson (10). Quantification was performed by means of densitometric scanning at 700 nm by using a thin-layer chromatography scanner II (Camag).

FA composition. Lipids were extracted and separated as described above. After nondestructive visualization of individ-
al PLs with primuline (65), the corresponding gel com-
partments as well as blanks were eluted after the addition of 10 μg pentadecanoic acid as an internal standard with 10 ml of chloroform-methanol (2:1 vol/vol). The ester bound FAs were converted to FA methyl ester (FAME) by using acid-

catalyzed transmethylation with 2 N HCl in methanol. For this purpose, samples (10–100 μg PL) were treated with 1 ml of reagent for 12 h at 100°C. Resulting FAME were extracted with 1 ml hexane, dried under a stream of nitrogen, and dissolved in 10 μl chloroform before further analysis. Gas chromato- 
graphic (Carlo Erba Fratovap 2150, Mainz, Ger-

dy) separation was performed as previously described (31) by using a polar-fused silica capillary column (CP-Sil 88, 50 m × 0.25 mm, Chrompack, Frankfurt, Germany) at 198°C, with helium as the carrier gas (flow rate, 1 ml/min). The injector was set at 250°C and used in a split mode (15:1). The individual FAME were detected by means of flame ion- 
zation detection and identified by comparison with the ret-
tention times of commercial standards (Sigma Chemical).

**RESULTS**

The analysis of BALF cells showed a significant drop in the relative amount of alveolar macrophages in all patient groups (Table 1). The number of neutrophils was elevated in IPF and HP, whereas the percentage of lymphocytes was markedly increased in patients with HP and Sarc.

The total PL concentration of BALF and the PL-to-
protein ratios of BALF as well as of LSA are shown in Table 2. We observed a slight and nonsignificant in-
crease in PLs of BALF but markedly and significantly decreased PL-to-protein ratios in all patient categories compared with controls. In contrast, LSA isolated from BALF by means of high-speed centrifugation showed no significant differences in the PL-to-protein ratios. Patients with IPF and Sarc had a lower relative con-
tent of LSA compared with controls; however, this decrease was not significant (Table 2).

Compared with controls (84.5%), the relative content of PC was significantly reduced in patients with IPF (78.3%, P < 0.05) but not in HP or Sarc (Table 2). Patients with IPF and HP revealed a markedly lower content of PG, ranging from <50% of control values in IPF patients. Therefore, the content of other minor PLs (PI, PE, PS, SPH) was elevated in IPF and, less pronounced, in HP. Altogether, the alterations in PL pro-
file were much more profound in patients with IPF than in HP, whereas the distribution of PL classes in...
Table 2. PL-to-protein ratios of BALF and LSA, relative content of LSA, and PL composition of BALF

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IPF</th>
<th>HP</th>
<th>Sarc</th>
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<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>12</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>PL BALF, µg/ml</td>
<td>31.69 ± 5.17</td>
<td>34.36 ± 7.81</td>
<td>47.22 ± 10.75</td>
<td>40.18 ± 3.81</td>
</tr>
<tr>
<td>PL/protein ratio of BALF</td>
<td>0.391 ± 0.059</td>
<td>0.207 ± 0.051*</td>
<td>0.176 ± 0.055†</td>
<td>0.252 ± 0.078*</td>
</tr>
<tr>
<td>PL/protein ratio of LSA</td>
<td>4.19 ± 0.46</td>
<td>3.91 ± 0.41</td>
<td>4.37 ± 0.47</td>
<td>4.94 ± 0.29</td>
</tr>
<tr>
<td>LSA, %total PL</td>
<td>73.5 ± 6.2</td>
<td>63.0 ± 4.8</td>
<td>70.5 ± 12.1</td>
<td>55.3 ± 8.4</td>
</tr>
<tr>
<td>PC, %</td>
<td>84.5 ± 0.8</td>
<td>78.3 ± 3.1a</td>
<td>83.9 ± 1.0</td>
<td>83.3 ± 1.3</td>
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<tr>
<td>PG, %</td>
<td>8.6 ± 0.8</td>
<td>3.5 ± 0.8†</td>
<td>6.4 ± 0.8</td>
<td>8.7 ± 0.9</td>
</tr>
<tr>
<td>PI, %</td>
<td>2.9 ± 0.3</td>
<td>7.4 ± 1.5b</td>
<td>5.1 ± 1.1*</td>
<td>2.5 ± 0.2</td>
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<tr>
<td>PE, %</td>
<td>1.5 ± 0.2</td>
<td>3.7 ± 1.7b</td>
<td>1.7 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>PS, %</td>
<td>0.6 ± 0.2</td>
<td>2.5 ± 1.1b</td>
<td>2.2 ± 0.6</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>SPH, %</td>
<td>0.8 ± 0.2</td>
<td>3.0 ± 0.8†</td>
<td>0.8 ± 0.3</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>LPC, %</td>
<td>0.0 ± 0.0</td>
<td>0.3 ± 0.2</td>
<td>0.6 ± 0.6</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>CL, %</td>
<td>1.1 ± 0.4</td>
<td>0.9 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>1.1 ± 0.3</td>
</tr>
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</table>

Values are means ± SE; n, no of subjects. Given is the phospholipid (PL) concentration in bronchoalveolar lavage fluid (BALF), the relative content of large-surfactant aggregates (LSA) (given in percentage of all BALF PL) and the PL-to-protein ratios (wt/wt), both in BALF and in LSA. The relative content of each PL is given in percentage of all PLs. PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PS, phosphatidylserine; SPH, sphingomyelin; LPC, lyso-PC; CL, cardiolipin. *P < 0.05; †P < 0.01 compared with controls.

Sarc did not substantially differ from that in healthy controls.

The FA profile of PC isolated from the LSA fraction is shown in Table 3. As anticipated, PC from controls was highly enriched in palmitic acid (∼80%), together with an overall content of saturated FAs of ∼88%. The predominant unsaturated FA was oleic acid (∼7.5%). Among patients, significant differences in the FA profile occurred only in IPF and comprised a marked loss of palmitic acid (∼67%; P < 0.01), paralleled by an increase in the relative amount of the unsaturated FAs, oleic acid (∼14%; P < 0.01), linoleic acid (∼6%; P < 0.001), and arachidonic acid. All other saturated FAs remained unchanged.

In additional experiments, we analyzed original BALF (containing both LSA and small-surfactant aggregates) for PC FA profiles. The changes in FA profiles of PC isolated from original BALF did not differ markedly from those changes observed in PC isolated from LSA. However, the relative concentration of palmitic acid was ∼10% lower in BALF PC compared with LSA PC, regardless of the observed category (data not given in detail). Accordingly, although performed in only a small number of patients and controls because of a shortage of material, we found a markedly reduced content of PC palmitic acid in small-surfactant aggregates compared with LSA (∼10–20% less). Together, these findings indicate a significantly lower relative content of PC palmitic acid in small-surfactant aggregates compared with LSA.

In healthy controls, PG and PI presented a very similar FA profile. Oleic acid was found to be the major FA in both PL species, followed by palmitic acid and stearic acid (Table 4). This similarity of FA composition of PG and PI was partially lost in IPF and, to a lesser extent, in HP. In both patient groups, the relative percentage of palmitic acid increased to ∼35% in PG but decreased in PI. This was paralleled by a significant decrease in the PG content of oleic acid, whereas the PI content in oleic acid did not change or was slightly increased. Further details are given in Table 4. The FA composition of PG and PI in Sarc remained largely unchanged compared with that in controls.

Because of the low concentration of other PLs such as PE, PS, and SPH in healthy subjects, we were not able to establish the FA profiles of these PLs in all cases, thus limiting statistical assessment because of a low number of subjects. The main FA of PE in controls was oleic acid (37.9 ± 2.8%), followed by palmitic acid (21.1 ± 5.7%), linoleic acid (14.0 ± 1.7%), stearic acid (13.3 ± 1.4%), and arachidonic acid (9.0 ± 1.7%; all n = 5). Among the patient groups, a more or less similar FA profile was observed.

Table 3. Fatty acid composition of PC isolated from LSA

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IPF</th>
<th>HP</th>
<th>Sarc</th>
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<tr>
<td>n</td>
<td>8</td>
<td>12</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>14:0</td>
<td>3.71 ± 0.72</td>
<td>4.69 ± 0.31</td>
<td>4.77 ± 0.69</td>
<td>3.51 ± 0.58</td>
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<tr>
<td>16:0</td>
<td>80.10 ± 1.73</td>
<td>66.84 ± 2.51†</td>
<td>78.47 ± 1.84</td>
<td>78.19 ± 3.11</td>
</tr>
<tr>
<td>18:0</td>
<td>4.13 ± 0.95</td>
<td>4.11 ± 0.69</td>
<td>2.65 ± 0.34</td>
<td>4.05 ± 1.02</td>
</tr>
<tr>
<td>16:1</td>
<td>1.79 ± 1.37</td>
<td>1.61 ± 0.59</td>
<td>3.00 ± 1.56</td>
<td>1.00 ± 0.53</td>
</tr>
<tr>
<td>18:1</td>
<td>7.49 ± 0.69</td>
<td>14.11 ± 1.65†</td>
<td>7.62 ± 0.99</td>
<td>10.45 ± 2.48</td>
</tr>
<tr>
<td>18:2</td>
<td>2.28 ± 0.37</td>
<td>5.93 ± 0.52‡</td>
<td>2.77 ± 0.66</td>
<td>3.14 ± 0.23*</td>
</tr>
<tr>
<td>20:4</td>
<td>0.49 ± 0.09</td>
<td>1.22 ± 0.35</td>
<td>0.45 ± 0.07</td>
<td>0.62 ± 0.27</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>87.95 ± 1.78</td>
<td>76.03 ± 2.23‡</td>
<td>86.00 ± 1.87</td>
<td>85.79 ± 2.97</td>
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</tbody>
</table>

Values are means ± SE; n, no of subjects. Depicted is the relative content of each fatty acid of PC in percentage of all fatty acids. Fatty acids with a relative distribution <0.5% are not given. 14:0, Myristic acid; 16:0, palmitic acid; 18:0, stearic acid; 16:1, palmitoleic acid; 18:1, oleic acid; 18:2, linoleic acid; 20:4, arachidonic acid. *P < 0.05; †P < 0.01; ‡P < 0.001 compared with controls.
The most abundant FA in SPH and PS was palmitic acid (~50%), followed by stearic acid and oleic acid (data not shown). Furthermore, SPH was composed of considerable amounts of long-chain saturated FAs (20:0, 22:0, 24:0; altogether ~15%).

The $\gamma_{\text{min}}$ of LSA from controls was found to be near 0 mN/m ($n = 8$). The $\gamma_{\text{min}}$ was moderately increased in HP (5.0 ± 2.3 mN/m; $n = 5$) and Sarc (3.8 ± 1.8 mN/m; $n = 10$) but dramatically increased in IPF (15.4 ± 2.7 mN/m; $n = 12$; $P < 0.001$ compared with control). As depicted in Fig. 1, the functionally most important feature in PL FA composition, the percentage in palmitic acid in the PC class, was significantly correlated with the $\gamma_{\text{min}}$ of the LSA fraction.

**DISCUSSION**

In the present study, patients with IPF displayed a markedly impaired surfactant function, with $\gamma_{\text{min}}$ values of ~15 mN/m, whereas controls reached values near 0 mN/m. The $\gamma_{\text{min}}$ of patients with HP (~5 mN/m) and Sarc (~3 mN/m) was only moderately increased. In addition to surfactant alterations that may be induced by inflammatory mediators (proteases, phospholipases), leaked plasma proteins, impaired balance of the alveolar surfactant subtype distribution, and alterations in SP content, changes in the biochemical composition of surfactant PL may offer one reasonable explanation for the impaired surface activity. In preceding studies, a moderate change in the PL pattern in IPF patients had been observed (15, 16, 29), comprising a reduction in the relative amount of PC and PG and a concomitant increase in PE, PI, and SPH. This profile of altered distribution of PL classes in IPF was fully corroborated in the present study, reaching statistical significance for all PL species. Interestingly, the same pattern of changes is known to occur in acute inflammatory lung diseases such as acute respiratory distress syndrome and severe pneumonia (8, 9, 11). Such changes were fully absent in Sarc, whereas in HP a minor tendency in this direction (e.g., decreased PG and increased PI percentage) was noted. In addition to the changes in PL species distribution, pronounced alterations in the FA profiles of PC, PG, and PI were demonstrated in IPF but were virtually absent in HP or Sarc. The observed differences between IPF patients and controls comprised mostly a loss of palmitic acid residues in PC and PI in favor of unsaturated FA and a decrease of stearic acid and oleic acid in favor of palmitic acid in PG. Our findings are in contrast to those of Baughman et al. (2), who reported a marked...
increase in the relative content of palmitic acid in PC of Sarc patients. In a small number of patients, Honda et al. (15) investigated the molecular species of PC and found decreased relative amounts of dipalmitoyl species in IPF, which favorably confirms our finding of a reduced percentage of saturated FAs, in particular, palmitic acid residues, in the PC fraction.

To what extent do these alterations in the FA profile of IPF patients contribute to the observed impairment of surface activity? A series of complex molecular interactions between the lipid components and the apoproteins of pulmonary surfactant is necessary for the extreme reduction of alveolar surface tension on expiration. In the presence of hydrophobic apoproteins, surface tension values near 0 mN/m on film compression may be achieved if the PLs, in particular PC, remain within the monolayer. Such a property is dependent on the phase transition temperature of the PC molecule, which in itself is dependent on the FA pattern. From previous in vitro studies, it is evident that the phase transition temperature of PC will fall with the degree of substitution with unsaturated FA species. On the other hand, a high degree of saturated, especially palmitic, residues will result in a higher phase transition temperature, and thus an improved film stability on lateral compression occurs. It is in line with this reasoning that in several in vitro studies very low \( \gamma_{\text{min}} \) values were only observed in the presence of very high relative amounts of DPPC (6, 13, 24, 25, 33). In addition, cyclic refinement of the interfacial monolayer may represent a second important property of pulmonary surfactant. Unsaturated molecular species of PC and acidic PLs are assumed to be excluded from the monolayer during film compression and are also assumed to readily reenter the film on film expansion (6, 12, 26). This high-adsorption capacity would require an adequately high proportion of unsaturated FAs. It is in line with such reasoning that the acidic PLs PG and PI are indeed characterized by a much higher percentage of unsaturated species compared with PC (14, 28). Against this background, both the reduction in palmitic acid residues in PC and the alterations in FA composition in PG (and to a lesser extent in PI) may well be relevant for the impairment of surface activity in patients suffering from IPF. A significant correlation between the \( \gamma_{\text{min}} \) and the percentage of palmitic acid residues in the PC class of patients with IPF was, indeed, noted. Although no proof has been established, such a close correlation suggests a functional significance of the alteration in PL FA composition for the impairment of surface activity in IPF patients.

What are the reasons for these profound alterations in PL FA profiles in IPF? One possible explanation would be contamination of the LSA with lipids from plasma or membrane constituents. This is, however, very unlikely in view of the different lipid profiles from these sources. For example, microsomes, plasma cell membranes, and macrophages contain a high proportion of PE (commonly ranging between 20 and 40% of all PL) (5, 22, 23). Furthermore, membrane-associated PI contains high amounts of polyunsaturated FAs (40–60%), mainly arachidonic acid (~30%), and relatively low amounts of oleic acid (~7%) (22, 23), which contrasts with the results obtained in this communication. Thus the data presented here suggest that disturbances in the alveolar type II cell metabolism may be primarily responsible for the alterations in surfactant PL and FA pattern. Possible mechanisms include 1) altered reuptake and intracellular sorting pathways, 2) a change in the acylation-deacylation cycle (“remodeling”) that is supposed to enrich DPPC (4, 27), or 3) alterations in the diacylglycerol pool that was incorporated in the PC de novo biosynthesis.

The underlying reasons why pronounced surfactant abnormalities appear in IPF, but only to a very minor extent in HP and virtually not in Sarc, are beyond the scope of the present study. There was no major difference in age, duration of disease, and smoking history among the different patient populations. Sarc patients were less dyspnoic and showed less restriction in lung function compared with IPF and HP. Furthermore, it may be questioned whether such findings are an underlying reason for, or a consequence of, the virtually full maintenance of normal surfactant characteristics in the Sarc population. Interestingly, lung function testing and dyspnea scores showed nearly identical limitations for IPF and HP patients, suggesting that the markedly more severe surfactant abnormalities in IPF are not a nonspecific consequence of the ongoing restriction and altered lung function but are a prominent aspect, especially in IPF.

In conclusion, in our study, employing a limited number of patients with ILD, biophysical characterization of the surfactant system revealed marked loss of surface tension-lowering capacities in IPF patients, compared with very moderate or near absence of changes in HP and Sarc. Coinciding with this difference in surfactant function, IPF patients were characterized by an altered distribution of surfactant PL classes, in particular, by a reduction in PC and PG and increased percentages of all minor PL species, and by marked changes in the FA profile of the major PL classes PC and PG. Whereas palmitic acid and overall saturated FA residues were significantly lowered in the PC fraction, the PG class showed lowered percentages of its major FA oleic acid at the expense of saturated FA. Changes in PC palmitic acid content were significantly correlated with loss in surface activity observed in patients with IPF. These data support an important role of surfactant abnormalities in the pathogenetic sequelae of IPF.

This study was supported by the Deutsche Forschungsgemeinschaft (SFB 547).
This paper includes parts of the doctoral thesis of U. Meier.

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