Allometry of the mammalian intracellular pulmonary surfactant system

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TERRESTRIAL MAMMALS SPAN A body mass range between a few grams in the Etruscan shrew and a few thousand kilograms in the elephant, i.e., six orders of magnitude. According to different metabolic needs, habitats, and living conditions, the structures that build up the organisms are economically designed to meet the functional demands, a phenomenon termed “symbiosis,” by Taylor and Weibel (51). Despite these differences, the similarities in organ design over such a great body mass range are remarkable. Both structural and functional characteristics (γ) can often be expressed as a function of body mass (M), providing a mathematical allometric relationship (27) of the form \( y = a M^b \), with the exponent \( b \) determining the slope of the curve and the intercept \( a \) determining the value if \( M = 1 \). The magnitudes of the latter, therefore, depends on the dimension of the parameter under investigation.

The lung as the principal organ of gas exchange is required to withstand the mechanical forces during the breathing cycle and to maintain a barrier between air and blood whose thickness equals a few hundred nanometers to a few micrometers and whose surface area equals many square meters in larger species, in case of the human lung, \( \sim 140 \) m\(^2\) (11, 29, 60). Studies on the allometry of the lung and its structural components have demonstrated that lung volume, alveolar epithelial surface area, and the numbers of the cells in the alveolar septa are closely correlated with body weight (13, 49).

The alveolar septa mainly consist of an epithelium (alveolar epithelial type I and type II cells) and a dense capillary network that is surrounded by stabilizing connective tissue that also contributes to the elastic properties of the gas-exchange region (reviewed in Ref. 37). The smooth liquid layer on top of the epithelium evokes a high surface tension, which would make the alveoli collapse during breathing (34). Therefore, a thin lining layer consisting of a complex mixture of \( \sim 90\% \) lipids and 10% proteins, the pulmonary surfactant, is located at the air-liquid interface (3, 14, 40). Surfactant not only lowers the surface tension at the air-liquid interface, but also protects the lungs against fluid influx from the underlying capillaries and participates in the innate immunity of the lung (for recent reviews, see Refs. 38, 41). Recently, it has been proposed that surfactant also facilitates the diffusion of oxygen across the air-blood barrier (39). Surfactant is produced, stored, secreted, and partly recycled by alveolar epithelial type II (AE2) cells and consists of an intra-alveolar and an intracellular pool (8). The main intracellular storing organelles, the lamellar bodies, are composed of densely stapled leaflets of surfactant material, which are separated from the cytosol by a limiting membrane; the total amount of lamellar bodies can thus be regarded as a morphological measure of the intracellular surfactant pool (reviewed in Ref. 38). Species differences exist with respect to the orientation of the lamellae, i.e., lamellae can be oriented in parallel or rather concentrically (50). Whether these major lamellar body subtypes correspond to different stages of lipid packing (41) is still under debate.

Although surfactant is essential for lung function, little is known about the relationships between body size, the size of the alveolar surface area, and the amount of surfactant in mammalian lungs. One might hypothesize that the intracellular surfactant pool size is similarly correlated to body mass as alveolar surface area, because it provides the reserve to cover the epithelium. However, the turnover of intra-alveolar surfactant may depend on other variables, such as respiratory rate, and may, therefore, require greater intracellular surfactant pools in mammals with higher respiratory rates. Because of the inverse relationship between body mass and respiratory rate (6, 47), one might argue that smaller mammals need a greater surfactant pool than larger animals, as related to their body mass. Similarly, size and curvature of alveoli greatly differ between species and might require a greater amount of surfactant (per unit body mass) in smaller species. Stone et al. (49) demonstrated that the number of AE2 cells scales with body

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mass and alveolar surface area, but did not investigate the amount of surfactant-storing lamellar bodies.

We hypothesized that the size of the intracellular surfactant pool is similarly correlated with body mass as the number of AE2 cells, but that the amount of lamellar bodies within individual AE2 cells is similar among terrestrial species, despite great variations in body size. Using lung tissue from 12 mammalian species spanning a body weight range of five orders of magnitude, we employed design-based stereology to quantify the size of the intracellular surfactant pool and the quantitative characteristics of AE2 cells.

MATERIAL AND METHODS

Animal and human lungs. This study was carried out on archive material from the Institute of Anatomy at the University of Bern, Switzerland, that was kindly provided by Professor Ewald R. Weibel. The following 12 species entered the stereological investigation. Etruscan shrew, mouse, rat, guinea pig, rabbit, dog, goat, human, camel, giraffe, steer, and horse. All lungs used in this study were previously investigated in a number of research projects. Details on the origin of the lungs and the fixation procedure are given in the corresponding articles (Table 1). All of the original studies from which the samples for this study are taken underwent a bioethical evaluation and were approved by the respective authorities. In case of the human lungs, the bioethical committee of the University Hospital of the University of Bern approved the original study by Gehr et al. (11).

In short, animals were deeply anesthetized or painlessly killed before lung fixation. All lungs were brought to collapse and subsequently fixed by airway instillation of 2.5% glutaraldehyde buffered to pH 7.4 with potassium-phosphate using a hydrostatic pressure of ~20–30 cmH2O, depending on the point of reference (sternum, chest, tracheal bifurcation). The human lungs were obtained, between 1971 and 1976, from subjects who had died from serious cerebral injuries, except for one case who had died from cardiac arrest. In compliance with current standards for human subjects, the lungs were de-identified by giving them numbers. Of the eight lungs reported by Gehr et al. (11), we arbitrarily picked five for this study. The human lungs were also fixed by instillation of the same fixative as used for the animals. After estimation of the reference volume by fluid displacement (43), the lungs were sampled using a stratified sampling scheme, i.e., randomly chosen tissue blocks were taken only from the parenchymal region of the lung. This procedure was similar in all investigated species and ensured that every part of the lung parenchyma had an equal chance of being analyzed. The samples were embedded in epoxy resin according to standard procedures (reviewed in Ref. 31), including postfixation with osmium tetroxide, en bloc staining with uranyl acetate, and dehydration in an ascending ethanol series. The volume of the lungs was multiplied by 0.85 to obtain the volume of the parenchyma, as the volume fraction of the parenchyma shows relatively little species variations with average values between 80 and 90% (42).

Stereology. From each animal, three different lobes were selected arbitrarily, and one tissue block per lobe was taken randomly and sectioned using an ultramicrotome. This sampling procedure and the relatively small number of three tissue blocks per animal can be considered to be sufficient to ensure that the sampling procedure did not dominate the variation between the analyzed groups because of the large differences between the species.

From each tissue block, a consecutive row of semithin sections (each 1 μm thick) was cut, and the first and fourth sections were mounted on one glass slide to generate a physical disector with a height of 3 μm (36). The sections were then stained with toluidine blue. Afterwards, from each tissue block, ultrathin sections (50 to 70-nm thick) were cut, mounted on formvar-coated copper support grids, and stained with uranyl acetate and lead citrate. For light microscopy, an Olympus BX51 light microscope (Olympus, Hamburg, Germany) was used, equipped with an Olympus DP72 digital camera and a computer with the newCAST software (Visiopharm, Horsholm, Denmark). Transmission electron microscopy was carried out with an EM 906 (Zeiss, Oberkochen, Germany) equipped with a slow-scan 2K charge-coupled device camera (TRS, Moorweins, Germany). Test fields for stereological analysis were gathered by systematic uniform random sampling (17).

At the light microscopic level, the surface area of alveolar epithelial cells was estimated by the intersection counting method (20, 58) at an objective lens magnification of ×40. A test system consisting of 14 line segments (length l = 22.18 μm) was projected onto each field of view, and the number of intersections (I) of the segments with the alveolar epithelium was counted, as well as the number of points (P) (defined as the left end of each line segment) hitting the gas-exchange region. From these counts, the surface density of the alveolar epithelium [Sv(alv/par)] was calculated according to $S_{v}(\text{alv}/\text{par}) = 2I/(lP)$. The number and the number-weighted mean volume of AE2 cells were estimated by the physical disector and the rotator, respectively (18, 48, 53), at an objective lens magnification of ×40. An unbiased counting frame was projected onto the corresponding fields of view from each section, and AE2 cells were counted if their nucleus was present in one of the two sections, but not in the other one (for a review, see Ref. 33). The numerical density of AE2 cells [$N_{v}(\text{AE2}/\text{par})$] was then calculated by $N_{v}(\text{AE2}/\text{par}) = Q^{-1}/v(\text{dis})$, where $Q^{-1}$ is the sum of all counted AE2 cells and $v(\text{dis})$ is the total volume of the disector. Additionally, if an AE2 cell was counted, it was simultaneously sampled for volume estimation by the rotator. The arithmetic mean of these estimations provided the number weighted mean volume of AE2 cells [$v_{\text{m}}(\text{AE2})$]. The surface density of the alveolar

| Table 1. Overview of the numbers of animals per species, mean body weight, and lung volume |
|-------------------------------|-----------------|-----------------|-----------------|
| Species                        | n               | Body Mass, g    | Lung Volume, ml |
| Etruscan shrew                 | 3               | 2.67 ± 0.37     | 0.103 ± 0.025   |
| Mouse                         | 5               | 41.7 ± 2.20     | 1.364 ± 0.056   |
| Rat                           | 3               | 308 ± 21        | 8.49 ± 0.83     |
| Guinea pig                    | 5               | 472 ± 12        | 15.2 ± 0.94     |
| Rabbit                        | 6               | 3,558 ± 1,263   | 79.2 ± 29.8     |
| Dog                           | 5               | 25,960 ± 2,740  | 1,408 ± 161     |
| Goat                          | 5               | 27,660 ± 6,694  | 1,742 ± 320     |
| Human                         | 5               | 79,800 ± 10,733 | 4,380 ± 950     |
| Camel                         | 2               | 231,750 ± 3,889 | 15,900 ± 1,980  |
| Giraffe                       | 1               | 383,000         | 21,000          |
| Steer                         | 3               | 473,667 ± 21,007| 25,467 ± 1,753  |
| Horse                         | 3               | 446,667 ± 61,889| 46,467 ± 6,261  |

Values are means ± SD; n, no. of animals. The last column indicates from which references the samples used in the present study originate and the respiratory rate data were taken from, respectively. Body mass and lung volume were taken from the respective references.
epithelium and the numerical density were multiplied by the volume of the parenchyma to provide the total surface area and the total number.

At the electron microscopic level, the volume density of the subcellular AE2 cell compartments “lamellar bodies,” “nucleus,” “mitochondria,” and “residual” was estimated by the point-counting method (58) at a primary magnification of ×7,750. A test system consisting of 72 points was projected onto each test field, and points hitting one of the four compartments \( P(\text{comp}) \) were counted. The volume density of a compartment \( VV(\text{comp}/\text{ref}) \) was calculated according to \( VV(\text{comp}/\text{ref}) = P(\text{comp})/P(\text{ref}) \), where \( P(\text{ref}) \) is the sum of all points hitting AE2 cells. The surface density of lamellar bodies was estimated at the same magnification using the intersection counting method, as described above. The volume-to-surface ratio, which is a useful parameter to evaluate the size of lamellar bodies (58), was calculated by dividing the volume density of lamellar bodies by their surface density. The volume-weighted mean volume of lamellar bodies \( V(Lb) \) was estimated by the point-sampled intercepts method (16), according to the formula \( V(Lb) = \left( \pi/3 \right) l_0^3 \) where \( l_0 \) is the mean edge-to-edge chord length of a lamellar body profile along

![Figure 1. Alveolar epithelial type II (AE2) cells from Etruscan shrew (A), rat (B), guinea pig (C), dog (D), human (E), and camel (F). Scale bar = 3 μm.](image-url)
a line intercept passing through a sampling point hitting the lamellar body profile. As the measurements are based on a volume-weighted sampling scheme, this parameter combines information on the size of lamellar bodies and on their size variation.

Statistics. Statistics were performed using SPSS 18.0. Means were tested for significant differences using Kruskal-Wallis-ANOVA and subsequent Mann-Whitney U-test. Due to the small number of some animal species, pairwise statistical testing was not applicable in several cases. Therefore, we chose not to include a labeling of statistical significance in Tables 1 and 2. All obvious differences between the species can be regarded as being biologically relevant, although, in some cases, the nature of the U-test precluded the differences from reaching significance level. The allometric relationships were expressed in the form of $y = ax^b$ using the module curve fit in the SPSS software, and regression analysis was performed to obtain the regression coefficient $R^2$. The nonlinear correlation between the stereological estimates and body mass/respiratory rate was carried out with Spearman’s rho test, which provided a coefficient of correlation ($r$) and a measure of statistical significance ($P$). The respiratory rate data used for some of the regression and correlation analyses were taken from the literature (7, 21, 24, 25, 46, 56). Significance level was $P < 0.05$.

RESULTS

Figure 1 shows the ultrastructure of AE2 cells for six of the investigated species. In all species, two types of lamellar bodies were observed with either parallel or concentric arrangement of the lamellae. While a parallel arrangement of the lamellae was a rare event in the human lung where concentrically arranged lamellae dominated, ~80% of the lamellar body volume of all the other investigated species showed parallel lamellae, and only 20% were of the concentric type. The packing density of the lamellar bodies appeared to be similar in all species, although this was hard to evaluate because of differences in fixation quality. No further consistent differences (apart from size, which was quantitatively evaluated) between the lamellar bodies were observed between the species.

The estimates of alveolar surface area ranged from 0.01 m$^2$ in the Etruscan shrew to over 1,000 m$^2$ in the steer and were similar to the data provided in the studies listed in Table 1. As such, the alveolar surface area correlated significantly with body mass and inversely with respiratory rate (Fig. 2). The total number of AE2 cells showed a variation between $6.7 \times 10^5$ in the shrew and $1.7 \times 10^{11}$ in the horse. The correlation with body mass was significant and had a similar slope as the alveolar surface area. AE2 cell number scaled inversely with respiratory rate (Fig. 3). In contrast, the mean volume of the AE2 cells did not show significant correlations with body mass or respiratory rate and varied ~500–600 μm$^3$ in most species (Fig. 4). Three species exhibited an increased mean AE2 cell volume with values between 900 and 1,300 μm$^3$, namely the Etruscan shrew, the guinea pig, and the human lungs. The mean volume of lamellar bodies per AE2 cell ranged between 80 and 100 μm$^3$ in most species, but was higher in the same species that exhibited a higher AE2 cell volume, with the shrew by far showing the greatest values (>200 μm$^3$). This was mainly due to the increased AE2 cell volume in these species, because the volume fraction of lamellar bodies was similar to that of the other species. When the total volume of lamellar bodies was calculated from the mean lamellar body volume per AE2 cell and the total number of AE2 cells, a close allometric relationship became apparent. The slope of the curve was very similar as that observed for alveolar epithelial surface area. Again, an inverse relationship to respiratory rate became apparent (Fig. 5). The mean volume of the other compartments (nucleus, mitochondria, cytoplasm) per AE2 cell also did not show an allometric relationship (data not shown). The volume-to-surface ratio of the lamellar bodies varied between 0.15 and 0.19 μm in the camel and giraffe, respectively, and 0.43 μm in the dog (Fig. 6). The volume-weighted
mean volume correlated well with the volume-to-surface ratio. Neither of these values showed a significant relationship with body mass. Table 2 contains a summary of the stereological data estimated in this study for each species. Table 3 provides an overview on the correlation analysis between some of the stereological parameters and body mass/respiratory rate.

**DISCUSSION**

Terrestrial mammals have evolved to span a body mass range of six orders of magnitude. Functional adaptations owing to this diversity include higher basal metabolism per unit body mass, as well as faster respiratory and heart rate in small mammals compared with larger ones, among others (47). In a series of investigations, Weibel and coworkers (13, 51, 57, 59) studied the allometric relationships of several pulmonary structures, mainly of those related to the function of the lung as gas exchanger. Based on their findings, the slope of the power law equation for the maximal rate of oxygen consumption is less steep than the slope of the equation for pulmonary diffusion capacity and for alveolar surface area (60). The allometric relationship of alveolar surface area, however, was observed to equal that of the maximum rate of oxygen consumption in an earlier study (52). The inclusion of marine animals by Tenney and Remmers (52), particularly for larger sized species, was discussed to explain this contradictory finding (13).

In the present study, we investigated whether the total intracellular volume of pulmonary surfactant, morphologically defined as the total volume of lamellar bodies, is either simply related to the alveolar surface area, in which case the curves of surfactant volume and surface area should have similar slopes, or whether other parameters known to influence surfactant biology, such as respiratory rate, influence the total amount of surfactant. Our results demonstrate that the slopes of the power law equations, i.e., the exponent \( b \) in the general equation \( y = aM^b \), of alveolar surface area and total lamellar body volume are very similar. Thus a relatively constant volume of lamellar bodies per unit of alveolar surface area can be found ranging from 6 to 22 mm\(^3\)/m\(^2\). Although the range of these data appears relatively large, it should be kept in mind that our study comprises animals over a body mass range of five orders of magnitude, with very different living conditions and, in case of the wild animals, unknown age. For such a heterogeneous group of animals, the variation can be regarded as small, and it is most important that there be no systematic variation correlating with body mass. These results are in good accordance with the biochemical analyses obtained from lung homogenates from 11 different species (4).

The ratio between lamellar body volume and alveolar surface area was also calculated by Vidic and Burri (54) in the lungs of postnatal rats and found to be 22 mm\(^3\)/m\(^2\) on day 4 and 23 mm\(^3\)/m\(^2\) on day 21. Similar amounts of lamellar bodies during postnatal rat lung development were also reported by Schmiedl et al. (44). Many of the allometric relationships during postnatal lung development deviate from interspecific allometric relationships because of the onset of air breathing and continued maturation of the lung (61). It is, therefore, interesting to note that the intracellular surfactant pool size seems to be in a close relationship with alveolar surface area both intra- and interspecifically. However, it should be mentioned that both size and volume of lamellar bodies in relation to alveolar surface area show considerable variation throughout postnatal development (44). Therefore, intra- and interspecific allometric relationships of total lamellar body volume should always be distinguished clearly. The small range of the lamellar body volume per unit alveolar surface area, as well as the relatively small size of the intracellular surfactant pool, appear to be possible only because of the relatively long turnover time of secreted surfactant, which is in the range of 1 to 10 h, depending on the study (62).

While answering the original question of this study, the finding is remarkable for the following reasons. The alveolar surface area of a human lung is \(~1,000\) times larger than in a mouse lung; however, the number of alveoli constituting this surface area is only 60 times larger in the human than in the mouse (23, 35). Thus the alveolar size is obviously smaller, and the curvature greater, in a mouse lung than in a human lung. Since the surface tension at the air-liquid interface increases with decreasing radius, according to Laplace’s law, the surface tension would be expected to be higher in the small alveoli of a mouse lung than in the larger alveoli of a human lung. Nevertheless, a species comparison of alveolar size and surface forces showed that, despite a fourfold difference in alveolar diameter between the mouse and the pig, the deflation stability and the mean surface tension are similar in 11 species, ranging from 0.03 to 50 kg body mass (28). One might argue...
that smaller mammals, therefore, need more surfactant per unit alveolar surface area than larger mammals. However, a distinction must be made between the surface-active intra-alveolar surfactant pool and the intracellular storage site of the lamellar bodies. It may indeed be possible that the volume of intra-alveolar surfactant per unit of alveolar surface area, and, therefore, the relation between intra-alveolar and intracellular pool size, differs between the mammals of different body size.

Due to the instillation fixation technique of the lungs used in this study, it was impossible to determine the volume of the intra-alveolar surfactant pool in the present study. Biochemical analyses have shown that the phosphatidylcholine (PC) content of lung tissue (after lavage) exceeds that in bronchoalveolar lavage fluid by a factor of two to three in mouse, rat, rabbit, and dog (10). However, later studies have provided evidence that the alveolar surfactant pool exceeds the intracellular surfactant pool greatly (63).

Postnatal development is often regarded as a special case of size allometry, because some parameters show a relationship with body mass similar to the interspecific comparisons, while other parameters do not. Interestingly, during postnatal development, the ratio between lamellar body volume and intra-alveolar phospholipids from lung lavage fluid increases from 0.47 mm$^3$mol at day 1 to 2.74 mm$^3$mol at day 42 in the rat lung. This is accompanied by differences in the content of surfactant protein A (44). These changes, however, are probably related to the development of the lung rather than to body mass allometry and, therefore, underline the difference between ontogenetic and interspecific allometric relationships. The volume of lamellar bodies per unit alveolar surface area can be increased by AE2 cell hyperplasia or hypertrophy, with the latter being much more effective than hyperplasia (30). Increased lamellar body volume per AE2 cell can be observed under chronic pathological conditions, e.g., after inhalation of pulmonary toxicants (30) or due to genetic mutation, e.g., surfactant protein D deficiency where AE2 cell hypertrophy is accompanied by hyperplasia (22).

As an alternative to the observation of this study, we had hypothesized that the intracellular surfactant pool might also be correlated with respiratory rate. Our data demonstrate that the number of AE2 cells, the total volume of lamellar bodies, and the alveolar surface area are inversely correlated with respiratory rate, which can be traced back to the allometric relationship of the respiratory rate. In fact, quantitative analyses of the fractions of the surfactant constituents dipalmityl-PC, palmitoyleoyl-PC, and palmitoilpalmitoleoyl-PC in different newborn and adult species have shown that the lipid composition of surfactant depends on pulmonary developmental status, respiratory rate (1), and species (26). Bernhard et al. (1) showed that both palmityloysteryl-PC and palmitylopalmitoleoyl-PC increased with increasing respiratory rate, whereas dipalmityl-PC rather decreased (1). Interestingly, Clements et al. (4) had already shown that the amount of dipalmityl-PC (in total lung tissue) was well correlated with the alveolar surface area, whereas the other phospholipids did not show a good correlation. These data support the concept that a relatively constant amount of surfactant, including its tissue reserve as lamellar bodies, is needed for covering the alveolar surface area, but that the composition of the surfactant is highly dependent on a variety of physiological demands (26).

Looking at from a clinical point of view, this might explain why exogenous surfactant therapy is beneficial in the respiratory distress syndrome of the neonate, but not of the adult. While the premature neonate has a primary lack of surfactant, the adult acute respiratory distress syndrome patient shows an altered composition of surfactant, making it dysfunctional.

Table 2. Summary of stereological data

<table>
<thead>
<tr>
<th>Species</th>
<th>$n$</th>
<th>$V_{\text{AEL}}$, m$^2$</th>
<th>$N$ (AE2, lung), $\times 10^6$</th>
<th>$v_{\text{V}}$(AE2), mm$^3$</th>
<th>$V_{\text{Lb}}$, lung, mm$^3$</th>
<th>$v_{\text{V}}$(Lb), mm$^3$</th>
<th>$V_{\text{Lb}}$-ratio(Lb), mm$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etruscan shrew</td>
<td>3</td>
<td>0.01 ± 0.004</td>
<td>0.66 ± 0.11</td>
<td>1304 ± 28</td>
<td>0.16 ± 0.02</td>
<td>1.06 ± 0.24</td>
<td>0.37 ± 0.06</td>
</tr>
<tr>
<td>Mouse</td>
<td>5</td>
<td>0.10 ± 0.001</td>
<td>7.45 ± 1.24</td>
<td>620 ± 75</td>
<td>0.71 ± 0.19</td>
<td>0.73 ± 0.32</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>Rat</td>
<td>3</td>
<td>0.45 ± 0.05</td>
<td>54 ± 4.6</td>
<td>610 ± 4</td>
<td>5.32 ± 0.51</td>
<td>0.84 ± 0.12</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>5</td>
<td>0.88 ± 0.17</td>
<td>130 ± 39.96</td>
<td>942 ± 76</td>
<td>18.59 ± 6.38</td>
<td>2.26 ± 0.50</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>Rabbit</td>
<td>6</td>
<td>4.18 ± 1.44</td>
<td>422 ± 16.8</td>
<td>622 ± 95</td>
<td>39.94 ± 18.63</td>
<td>1.50 ± 0.87</td>
<td>0.38 ± 0.05</td>
</tr>
<tr>
<td>Dog</td>
<td>5</td>
<td>48.6 ± 6.0</td>
<td>6,762 ± 2,315</td>
<td>561 ± 54</td>
<td>848 ± 414</td>
<td>1.71 ± 0.58</td>
<td>0.43 ± 0.07</td>
</tr>
<tr>
<td>Goat</td>
<td>5</td>
<td>65.6 ± 9.5</td>
<td>6,596 ± 1,579</td>
<td>533 ± 68</td>
<td>558 ± 117</td>
<td>1.21 ± 0.39</td>
<td>0.35 ± 0.04</td>
</tr>
<tr>
<td>Human</td>
<td>5</td>
<td>112 ± 37</td>
<td>16,689 ± 5,189</td>
<td>915 ± 59</td>
<td>2,399 ± 628</td>
<td>0.80 ± 0.16</td>
<td>0.34 ± 0.08</td>
</tr>
<tr>
<td>Camel</td>
<td>2</td>
<td>430 ± 31</td>
<td>71,262 ± 5,684</td>
<td>614 ± 42</td>
<td>6,110 ± 1,093</td>
<td>0.62 ± 0.23</td>
<td>0.15 ± 0.05</td>
</tr>
<tr>
<td>Giraffe</td>
<td>1</td>
<td>850</td>
<td>105,400</td>
<td>588</td>
<td>7,562</td>
<td>0.70</td>
<td>0.19</td>
</tr>
<tr>
<td>Steer</td>
<td>3</td>
<td>951 ± 183</td>
<td>119,100 ± 18,160</td>
<td>549 ± 41</td>
<td>11,570 ± 5,286</td>
<td>1.46 ± 0.12</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>Horse</td>
<td>3</td>
<td>1,577 ± 677</td>
<td>167,300 ± 25,271</td>
<td>611 ± 72</td>
<td>14,187 ± 6,470</td>
<td>2.11 ± 0.69</td>
<td>0.23 ± 0.09</td>
</tr>
</tbody>
</table>

Values are means ± SD; $n$, no. of animals; $V_{\text{AEL}}$, total alveolar epithelial surface area; $N$ (AE2, lung), total number of alveolar epithelial type II cells; $v_{\text{V}}$(AE2), number-weighted mean volume of alveolar epithelial type II cells; $V_{\text{Lb}}$, total volume of lamellar bodies; $v_{\text{V}}$(Lb), volume-weighted mean volume of lamellar bodies; $V_{\text{Lb}}$-ratio(Lb), volume-to-surface ratio of lamellar bodies.

Table 3. Correlation analysis using Spearman’s rho test

<table>
<thead>
<tr>
<th>Correlation vs. Body Mass</th>
<th>$r$</th>
<th>$P$</th>
<th>Correlation vs. Respiratory Rate</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE surface area</td>
<td>1.0</td>
<td>&lt;0.01</td>
<td>AE surface area</td>
<td>−0.87</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Number of AE2 cells</td>
<td>0.986</td>
<td>&lt;0.01</td>
<td>Number of AE2 cells</td>
<td>−0.874</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Volume of Lb</td>
<td>0.986</td>
<td>&lt;0.01</td>
<td>Volume of Lb</td>
<td>−0.874</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean volume of AE2 cells</td>
<td>−0.538</td>
<td>0.071</td>
<td>Mean volume of AE2 cells</td>
<td>0.425</td>
<td>0.169</td>
</tr>
<tr>
<td>Volume-to-surface ratio of Lb</td>
<td>0.332</td>
<td>0.297</td>
<td>Volume-to-surface ratio of Lb</td>
<td>0.425</td>
<td>0.165</td>
</tr>
</tbody>
</table>

The $r$ value provides information on the quality of the correlation, whereas the $P$ value indicates the statistical significance of the correlation. AE, alveolar epithelial.
rather than an amount too low to cover the alveolar surface area (32, 45).

Apart from these general relationships, some interesting exceptions were found, with the Etruscan shrew showing the greatest differences to the other species. In the Etruscan shrew, AE2 cells are particularly often found to open into two or even three alveoli (Fig. 1A), which may explain why they are so big. The physiological aim of these big cells may be to keep the number of AE2 cells (which clearly represent some of the biggest parts of the air-blood barrier) low. A larger AE2 cell volume and, hence, a larger volume of lamellar bodies per AE2 cell was also found in guinea pig and human. An interpretation of these differences would remain very speculative.

Taken together, the mean volume of AE2 cells, as well as their subcellular composition, appear to be independent of body mass, although some species differences exist. Thus the adaptation of the intracellular surfactant pool size is obtained by increasing the number of AE2 cells with increasing body mass, but not the volume of lamellar bodies per AE2 cell. Under normal conditions, the size of the intracellular surfactant pool is a relatively constant parameter, both intra- and inter-specifically. Adaptations to varying physiological demands, such as changes in respiratory rate or size of alveoli, might rather depend on the biochemical composition of the surfactant system.

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DISCLOSURES

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