Effect of surface tension of mucosal lining liquid on upper airway mechanics in anesthetized humans

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Effect of surface tension of mucosal lining liquid on upper airway mechanics in anesthetized humans. J Appl Physiol. 95: 357–363, 2003. First published March 7, 2003; 10.1152/japplphysiol.01198.2002.—Upper airway (UA) patency may be influenced by surface tension (γ) operating within the (UAL). We examined the role of γ of UAL in the maintenance of UA patency in eight isoflurane-anesthetized supine human subjects breathing via a nasal mask connected to a pneumotachograph attached to a pressure delivery system. We evaluated 1) mask pressure at which the UA closed (Pcrit), 2) UA resistance upstream from the site of UA collapse (RUS), and 3) mask pressure at which the UA reopened (Po). A multiple pressure-transducer catheter was used to identify the site of airway closure (velopharyngeal in all subjects). UAL samples (0.2 μl) were collected, and the γ of UAL was determined by using the “pull-off force” technique. Studies were performed before and after the intrapharyngeal instillation of 5 ml of exogenous surfactant (Exosurf, Glaxo Smith Kline). The γ of UAL decreased from 61.9 ± 4.1 (control) to 50.3 ± 5.0 mN/m (surfactant; P < 0.02). Changes in Po, RUS, and P – Perit (change = control – surfactant) were positively correlated with changes in γ (r² > 0.6; P < 0.02) but not with changes in Perit (r² = 0.4; P > 0.9). In addition, mean peak inspiratory airflow (no flow limitation) significantly increased (P < 0.04) from 0.31 ± 0.06 (control) to 0.36 ± 0.06 l/s (surfactant). These findings suggest that γ of UAL exerts a force on the UA wall that hinders airway opening. Instillation of exogenous surfactant into the UA lowers the γ of UAL, thus increasing UA patency and augmenting reopening of the collapsed airway.

SURFACE FORCES associated with the liquid lining the upper airway (UAL) may play a role in determining the mechanical properties of the upper airway (UA), especially in regard to the magnitude of pressures required to separate mucosal surfaces that come into contact during airway collapse (18). In animals, surface-active agents instilled into the UA reduce airflow resistance (26) and, in both our laboratory’s (5, 13) and other studies (15, 18), decrease UA intraluminal pressures associated with airway closure (closing pressure) and reopening (opening pressure). Our laboratory has also previously shown that the UA in awake, healthy humans is more resistant to narrowing and collapse after topical application of a synthetic lung surfactant preparation (23). Jokic and coworkers (10) demonstrated that addition of a topical lubricant to the UA significantly reduced the severity of sleep-disordered breathing events in patients with obstructive sleep apnea/hypopnea syndrome (OSAHS). Recently, Morrell et al. (16) have shown in OSAHS patients that instillation of an exogenous surfactant into the UA decreases both pharyngeal airflow resistance and the rate of occurrence of apneas and hypopneas during sleep. Although these studies give some insight into the mechanisms of UA collapse, none provide any direct measurement of UA surface forces. Consequently, the precise nature of these surface forces, the magnitude of their contribution to airway collapse and reopening, and their potential for therapeutic modification remain undefined.

Recently, our laboratory (13) demonstrated in anesthetized rabbits that there was a significant positive relationship between the surface tension (γ) of the UAL and UA closing and opening pressures, but there are no comparable data in humans. However, Eastwood et al. (7, 8) have also recently shown that state-related inhibition of motoneuron activity during general anesthesia in humans allows UA mechanics to be studied in the absence of UA muscle activity. This approach allows assessment of UA collapsibility without the confounding influence of reflex neurogenic activity. In the present study, we utilize this anesthetized human model to examine the effect of modifying γ of UAL on the mechanical properties of the UA in adult human subjects.

METHODS

Subject selection. Eight participants (2 men, 6 women; see Table 1 for anthropometric data) were selected from patients...
undergoing general anesthesia for minor surgery not involving the head or neck. There was no a priori determination of the propensity for subjects to have sleep-disordered breathing or respiratory disease. The study was approved by the Sir Charles Gairdner Hospital Ethics Committee, and informed consent was obtained from each participant.

**Subject preparation.** The methodology used in the present study is based on the approach described by Eastwood et al. (7). Briefly, anesthesia was induced with intravenous propofol (1.5–2 mg/kg) and maintained during surgery with isoflurane and nitrous oxide in oxygen, which were administered via a laryngeal mask. A four-sensor pressure transducer catheter (Gaeltec CTO-4; Dunvegan, Isle of Skye, Scotland) was passed via the nares into the UA and esophagus to simultaneously measure esophageal (Pes), hypopharyngeal, oropharyngeal, and nasopharyngeal pressures. A similarly placed polyethylene catheter, positioned with its tip in the oropharynx and connected to a gas analyzer (model 602, Poet II, Criticare Systems, Waukesha, WI), was used to continuously monitor end-tidal carbon dioxide and isoflurane particle pressure (PETiso) levels. Genioglossus electromyographic activity (EMGgg) was measured with intramuscular electrodes inserted percutaneously according to methods previously described (7). On completion of their surgical procedure, each patient was then placed supine with their head in a neutral position. The laryngeal mask was then removed, the mouth taped closed, and a nasal mask fitted, via which anesthesia was maintained by using isoflurane (0.8%) in oxygen. The anesthesia system was modified to provide control over the nasal mask pressure (Pmask), which could be adjusted to provide differing levels of continuous positive airway pressure (CPAP) or a subatmospheric pressure. Airflow was monitored with a pneumotachograph (model 47903A, Hewlett Packard, Waltham, MA) connected to the nasal mask. All signals were digitally recorded with a sampling frequency of 1,000 Hz on a PowerLab data acquisition and analysis system (model 16s; ADInstruments, Sydney, Australia) and stored on a computer for later analysis.

**Measurement of airway closure.** The collapsibility of the UA was assessed by measuring the Pmask associated with the cessation of respiratory airflow (Pcrit), as described by Smith et al. (22). Intermittently, Pmask was rapidly decreased from a maintenance level (Fig. 1) that was sufficient to prevent inspiratory airflow limitation. Inspiratory airflow limitation was identified by the presence of an inspiratory flow plateau associated with continuing fluctuations in Pes. This lower Pmask level was determined by the appearance of inspiratory airflow limitation and maintained for five successive breaths before being returned to the maintenance level. This procedure was repeated (3–5 runs) to obtain a range of Pmask and peak inspiratory airflow (V_{imax}) values during airflow limitation (Fig. 2). For the runs where there was airflow limitation, only breaths 3, 4, and 5 of the five successive breaths were used for further analysis (7) (see also Data analysis below).

### Table 1. Subject anthropometric data

<table>
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<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Gender</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
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<td>F</td>
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<td>Mean ± SE</td>
<td>45.2 ± 2.0</td>
<td>167 ± 5</td>
<td>88 ± 7</td>
<td>31 ± 2</td>
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BMI, body mass index; F, female; M, male.

**UA samples.** UAL samples (−0.2 μl) were collected with a catheter (outer diameter = 2 mm; inner diameter = 1 mm) attached to a 1-ml syringe and advanced via the nares into the posterior pharyngeal lumen. Samples were taken from the mucosal lining liquid in the UA at the site of UA collapse (see Data analysis below).

**Data analysis.** As previously described by Smith et al. (22), Pcrit was calculated from the relationship between Pmask and Peak Inspiratory Airflow (V_{imax}) values during airflow limitation (Fig. 2). For the runs where there was airflow limitation, only breaths 3, 4, and 5 of the five successive breaths were used for further analysis (7) (see also Data analysis). The collapsibility of the UA was assessed by measuring the Pmask associated with the cessation of respiratory airflow (Pcrit), as described by Smith et al. (22). Intermittently, Pmask was rapidly decreased from a maintenance level (Fig. 1) that was sufficient to prevent inspiratory airflow limitation. Inspiratory airflow limitation was identified by the presence of an inspiratory flow plateau associated with continuing fluctuations in Pes. This lower Pmask level was determined by the appearance of inspiratory airflow limitation and maintained for five successive breaths before being returned to the maintenance level. This procedure was repeated (3–5 runs) to obtain a range of Pmask and peak inspiratory airflow (V_{imax}) values during airflow limitation (Fig. 2). For the runs where there was airflow limitation, only breaths 3, 4, and 5 of the five successive breaths were used for further analysis (7) (see also Data analysis). As previously described by Smith et al. (22), Pcrit was calculated from the relationship between Pmask and V_{imax} as the Pmask at which V_{imax} became zero.
Measurement of airway reopening. Airway opening pressure (Po) was measured by decreasing Pmask to 1 cmH2O below Pcrit for two inspiratory efforts (monitored by Pes) before Pmask was increased for the third inspiratory effort. Pmask was then returned to the maintenance pressure immediately after the third inspiratory effort. This cycle was repeated with the pressure level for the third inspiratory effort being incremented by 0.5 cmH2O for each successive cycle until a Pmask was reached where respiratory airflow was reestablished (Fig. 3). Po was taken as the average third-breath Pmask for the last two cycles in the series.

Genioglossus muscle activity. At the conclusion of the data-gathering phase of the study, a square-wave negative pressure of −30 cmH2O was applied to the airway for a single inspiratory effort to determine whether a reflex response could be obtained from the genioglossus muscles (i.e., an increase in EMGgg).

Measurements of γ. Measurements of γ were made by using the "pull-off force" technique described by Kirkness et al. (12). In this method, the force required to separate two silica surfaces bridged by the test liquid sample is used to measure the γ of the sample. This method permits measurements of γ to be performed with sample volumes as small as ~0.2 μl.

Exogenous surfactant. The γ of UAL was altered by instilling an exogenous surfactant into the UA. Five milliliters of Exosurf Neonatal (Exosurf Neonatal, Glaxo Smith Kline, Greenville, NC) was instilled via a polyethylene catheter (outer diameter = 2 mm; inner diameter = 1 mm) advanced through the nares to the posterior pharynx. Exosurf Neona-

Fig. 2. Airflow limitation. Section of the same data from Fig. 1 showing V̇, Pmask, and Pes for breaths 3, 4, and 5 for control data for runs #C to #E. Upper airway collapsibility was assessed from breath-by-breath mean peak inspiratory V̇ and Pmask data (arrows). See text for explanation. Note that for run #E respiratory efforts are present (see Pes), but there is no resultant V̇, indicating closure of the upper airway.

Fig. 3. Airway opening pressure. A: Pmask is reduced below critical pressure (Pcrit), and the airway closes (i.e., no respiratory-related phasic fluctuations in V̇). After 2 respiratory efforts, Pmask is increased (+ΔPmask); however, the airway remains closed. B: Pmask is increased in 0.5-cmH2O incremental steps (+ΔPmask), the airway reopens, and phasic V̇ returns. Pmask at which the upper airway reopened (Po) was measured as the Pmask required to reestablish respiratory V̇.
al is stored as a sterile white lyophilized powder in vacuum vials. Each vial contains 108 mg of colfosceril palmitate formulated with 12 mg of cetyl alcohol, 8 mg of tyloxapol, and 47 mg of sodium chloride. When reconstituted with 8 ml of sterile water, Exosurf neonatal suspension contains 13.5 mg/ml colfosceril palmitate, 1.5 mg/ml cetyl alcohol, and 1 mg/ml tyloxapol in 0.1 M NaCl, and it has an osmolality of 183 mosmol/kg H2O. The γ of Exosurf Neonatal has been reported to be between 38 and 44 mmHg (1, 20).

Protocol. With the subject instrumented and PetIso maintained at 0.8%, a maintenance Pmask (6–17 cmH2O) was applied at a level sufficient to ensure no inspiratory airflow limitation. Once a stable baseline was established, a measurement of Perit was obtained followed by a measurement of Po. UAL samples were then obtained prior to instillation of 5 ml of Exosurf into the nasopharynx. Measurements of Perit, Po, and γ of UAL were then repeated.

After completion of the measurements of UA mechanics, a square-wave negative pressure of −30 cmH2O was applied to the airway. Inspired isoflurane was then discontinued, and the subject recovered from anesthesia while breathing 100% oxygen via the nasal mask at the maintenance CPAP level. Inspired iso-urethane was then discontinued, and recovery of consciousness was allowed to proceed. The patient was allowed to recover consciousness.

Data analysis. In addition to Perit and Po values, RUS was calculated from the slope of the UA pressure-flow relationship (Fig. 4) obtained from breaths 3, 4, and 5 during airflow limitation (2, 22). The site of UA collapse (nasopharyngeal, oropharyngeal, or hypopharyngeal) was determined from the transmission of respiratory-related pressure fluctuations along the UA as determined from the four sensor pressure transducer catheter signals. The difference between Po and Perit was calculated as Po − Perit. Values are reported as means ± SE. Pre- and post-surfactant group mean data for γ of UAL, Po, Perit, Po − Perit, RUS, and PetIso were compared by using Student’s paired t-test. The relationship between the change in γ of UAL and the change in UA mechanics measures was examined by using linear regression analysis. P < 0.05 was considered significant.

RESULTS

Depth of anesthesia. Control and surfactant data were obtained under similar levels of anesthesia, as determined by the level of PETIso (control: 0.84 ± 0.04%; surfactant: 0.85 ± 0.03%; P = 0.6).

Genioglossus muscle activity. No phasic respiratory-related EMGgg activity was detected in any subject for any condition throughout the study, including during the inspiratory efforts that occurred with the application of −30 cmH2O to the mask. In all subjects, phasic respiratory EMGgg signals were obtained within 5 min of removal of the anesthetic gas. During the recovery period, either swallowing and/or tongue protrusion generated a substantial EMGgg signal in all subjects.

γ of UAL. After administration of exogenous surfactant, the γ of UAL decreased in seven of the eight subjects (Fig. 5). For the group, γ of UAL decreased from 61.9 ± 4.1 (control) to 50.3 ± 5.0 mN/m (surfactant; P < 0.02).

UA mechanics. Typical responses to decreasing Pmask during general anesthesia in one subject are shown in Fig. 1. In all subjects, inspiratory flow limitation was apparent when Pmask was reduced sufficiently below the maintenance pressure. On each occasion that Pmask was reduced to a level sufficient to cause inspiratory airflow limitation for five successive breaths, Vlmmax progressively decreased over the first two breaths before stabilizing between breaths 3 and 5 (see Fig. 2). This pattern of change of Vlmmax was seen in all subjects during all sequences of flow-limited breaths. In each subject, Vlmmax was linearly related to Pmask for flow-limited breaths both before and after the administration of surfactant (see Fig. 4; all r² > 0.91; all P < 0.05). Control Perit values for subjects 4–6 were also included in data previously reported by Eastwood et al. (7).

In all subjects, the site of obstruction was located in the velopharynx. Figure 1 demonstrates a typical example where a period of zero flow was accompanied by similar changes in Pes, hypopharyngeal pressure, and oropharyngeal pressure during inspiratory efforts together with failure of these pressure changes to be transmitted to the nasopharynx, thus indicating retropalatal airway obstruction. The site of obstruction was not altered by administration of surfactant in any subject.

In four subjects, Perit decreased with the instillation of surfactant into the UA. Similarly, Po decreased in four subjects, RUS decreased in six subjects, and Po − Perit decreased in six subjects. However, despite these tendencies toward a change with surfactant, there were no significant group mean differences between control and surfactant conditions for Perit (3.3 ± 0.8 vs. 3.0 ± 0.8 cmH2O, respectively; P = 0.3), Po (0.4 ± 1.3 vs. 0.2 ± 1.2 cmH2O; P = 0.5), or RUS (17.4 ± 2.3 vs. 15.3 ± 1.5 cmH2O · l−1 · s; P = 0.3).

Fig. 4. Upper airway collapsibility. Individual subject data points (for breaths 3, 4, and 5 from runs #C, #D, and #E in Figs. 1 and 2) together with linear regression line and equations for peak inspiratory Pmask vs. peak inspiratory V (Vmax) during V limitation for control and surfactant conditions. x-Axis intercept was taken as the airway collapsing pressure (Perit). The inverse of the slope of the regression lines is a measure of the resistance of upper airway upstream from site of collapse (RUS).
Changes in UA mechanics vs. changes in γ of UAL. The change in Po (control − surfactant) after the administration of surfactant was strongly and positively correlated (r = 0.89; P < 0.003; Fig. 6) with the change in γ of UAL. Similarly, the change in RUS also strongly correlated with the change in γ (change in RUS: r = 0.93; P < 0.001; Fig. 6). However, changes in Pcrit with surfactant were not significantly correlated with the change in γ (r = 0.4; P > 0.9), although the change in Po − Pcrit was positively correlated with the change in γ (r = 0.8; P < 0.02).

CPAP related V̇Imax. Although there was no significant difference (P = 0.9) between the mean V̇Imax during control (11.2 ± 1.3 cmH2O) and surfactant (11.0 ± 1.2 cmH2O) conditions, mean V̇Imax (no airflow limitation) significantly increased (P < 0.04) from 0.31 ± 0.06 l/s during control to 0.36 ± 0.06 l/s during surfactant. Such an increase occurred in all but one of the subjects and is consistent with a fall in UA resistance associated with instillation of surfactant into the UA. The magnitudes of the changes in mean V̇Imax with surfactant, however, were not significantly correlated with changes in γ (r = 0.3; P > 0.4).

DISCUSSION

The major findings in this study of anesthetized humans were 1) topical instillation of an exogenous surfactant into the UA decreased the γ of the UAL from ~62 to ~50 mN/m; 2) these changes in γ were correlated with decreases in Po, Po − Pcrit, and RUS; and 3) the V̇Imax achieved at the same level of CPAP increased after the instillation of exogenous surfactant into the UA.

The values for γ measured in the present study are the first measurements reported in the literature for UAL in humans. At ~62 mN/m, the γ of human UAL is substantially less than that for saline [71.2 mN/m (14)], which reflects the presence of endogenous surfac-
tants in human UAL. Although UAL has not been previously studied in humans, there have been a number of previous studies examining the γ of saliva (3, 9). Saliva is 95% water but contains small concentrations of phospholipids with surfactant properties (6, 24). Reported values for the γ of human saliva range from 53.1 to 57.0 mN/m (3, 9). In the present study, the group mean value obtained for γ of UAL was slightly greater than this reported range. This may represent a difference between UAL and saliva or may be related to the conditions under which the samples were obtained (e.g., general anesthesia).

This is the first study to establish a quantitative relationship between the γ of UAL and passive mechanical properties of the human UA. The findings in this study are consistent with our laboratory’s previous findings in anesthetized rabbits (13). They are also compatible with published studies by our laboratory and others, in which instillation of surface active agents into the UA of healthy awake humans (23) and sleeping patients with OSAHS (10, 16) were associated with improved UA patency. Our findings suggest that the γ of UAL contributes importantly to the balance of forces acting across the UA wall. It acts to hinder airway opening when UA mucosal surfaces are in apposition and appears to increase RUS when the airway is patent. It is modifiable by instillation of exogenous surfactants into the UA lumen.

Modest levels of general anesthesia in human subjects are associated with profound depression of UA muscle tone and reflex motor activity (4, 7), as seen in this study. Eastwood and colleagues (7) have recently used this circumstance to study the passive (i.e., no EMG response to a negative UA intraluminal pressure stimulus) mechanical properties of the human UA (7, 8). Moreover, they have also recently shown that the mechanical properties of the UA during general anesthesia are related to its behavior during sleep (7).

The role of surface forces in maintenance of alveolar patency has been extensively investigated, but there are fewer investigations examining their role in maintenance of UA patency (5, 10, 13, 15, 16, 18, 23, 26). An important difference between these previous studies and the present one is that, with the exception of our laboratory’s study in rabbits (13), none of the former studies report actual measurements of the γ of UAL because most previous investigators have simply assumed that instillation of a surface-active agent into the UA will decrease γ of UAL. In the present study, we have directly measured the γ of UAL, thus allowing us to quantitatively relate the magnitude of changes in γ with instillation of exogenous surfactant to changes in UA mechanical properties. Our data do not rule out the possibility that effects on UA mechanics were not due to the change in the γ of UAL but were due to some other effect of surfactant. We, however, consider this unlikely because compatible findings have been reported by other investigators using different surfactants (11, 16).

The major effect of lowering the γ of UAL in anesthetized human subjects was to decrease Po. This finding is consistent with that of Van der Touw et al. (23), who found a substantial decrease in Po after instillation of exogenous surfactant into the UA of awake human subjects, and of our laboratory’s previous study in rabbits (13). Pcrit is a measure of UA collapsibility that has been used widely to assess UA mechanical properties in animals (17, 19) and humans (2, 21, 22). Values for Pcrit obtained in the present study ranged from −4.3 to +7.3 cmH2O under control conditions. These values are similar to those we have obtained previously (8) but are substantially greater than the value of approximately −12 cmH2O that was reported in awake subjects by Van der Touw and colleagues. This difference most likely reflects different levels of neuromuscular activity between the awake and anesthetized state. The wide range of Pcrit values encountered suggests that we were probably dealing with a heterogeneous group in terms of their UA mechanical properties.

Although Pcrit decreased with surfactant in four of eight subjects, this effect did not reach statistical significance for the group, nor was the change in Pcrit significantly correlated with the change in γ of UAL. This result contrasts with our laboratory’s previous finding in rabbits (13), where a range of changes in γ of UAL was achieved by administering both surfactant and saline, which tends to increase γ of UAL. Furthermore, UA closing pressure has been shown to decrease with gargling of exogenous surfactant in awake humans (23). Differences in the delivery and uniformity of distribution of the surfactant within the UA may explain these different findings. Although we studied only eight subjects, they conferred sufficient power (85%) to detect a difference of 1 cmH2O. Hence, although we cannot exclude the possibility that instillation of surfactant decreases Pcrit, we believe that any such change is likely to have been small.

Our findings suggest that changing γ of the UAL has less impact on airflow closure than airflow reopening. Previous studies have consistently demonstrated a difference between UA opening and closing pressure in both animal (5) and human studies (23, 27). As our laboratory has previously demonstrated in rabbits (13), change in Po − Pcrit was positively correlated with change in γ of UAL. Moreover, this relationship explained 60% of the variance in Po − Pcrit, indicating that γ of UAL has a major influence on the difference between UA closure and reopening pressures.

A further finding in the present study was the relationship between change in RUS and change in γ of UAL. This is consistent with previous findings in dogs (26) and sleeping OSAHS patients (16) and also with the work of Ward et al. (25), who showed that application of a topical lubricant into the UA decreased the CPAP pressure requirement of patients with severe OSAHS. It would appear that the γ of UAL influences UA mechanics not only at the point of closure and reopening but also when the airway is patent. Our finding that airflow increased after surfactant administration, at the same level of CPAP, is supportive of this concept.
In summary, we have demonstrated that decreasing the γ of UAL in anesthetized humans reduces UA airflow resistance and augments reopening of the collapsed UA. We conclude that, at least in states associated with depression of neuromuscular activity, such as general anesthesia and sleep, forces associated with the UAL act to reduce UA patency. This influence on UA mechanics, however, is modifiable through the instillation of exogenous surfactant into the UA.

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REFERENCES