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Decreased surface tension of upper airway mucosal lining liquid increases upper airway patency in anaesthetised rabbits

Jason P. Kirkness, Hugo K. Christenson*, Sarah R. Garlick, Radha Parikh, Kristina Kairaitis, John R. Wheatley and Terence C. Amis

*Ludwig Engel Centre for Respiratory Research, Westmead Hospital and University of Sydney, New South Wales, Australia, 2145 and *Department of Physics and Astronomy, The University of Leeds, Leeds LS2 9JT, UK*

The obstructive sleep apnoea syndrome (OSA) is a disorder characterised by repetitive closure and re-opening of the upper airway during sleep. Upper airway luminal patency is influenced by a number of factors including: intraluminal air pressure, upper airway dilator muscle activity, surrounding extraluminal tissue pressure, and also surface forces which can potentially act within the liquid layer lining the upper airway. The aim of the present study was to examine the role of upper airway mucosal lining liquid (UAL) surface tension (γ) in the control of upper airway patency. Upper airway opening (P_O) and closing pressures (P_C) were measured in 25 adult male, supine, tracheostomised, mechanically ventilated, anaesthetised (sodium pentabarbitone), New Zealand White rabbits before (control) and after instillation of 0.5 ml of either 0.9 % saline ($n = 9$) or an exogenous surfactant ($n = 16$; Exosurf Neonatal) into the pharyngeal airway. The γ of UAL (0.2 μl) was quantified using the 'pull-off' force technique in which γ is measured as the force required to separate two curved silica discs bridged by the liquid sample. The γ of UAL decreased after instillation of surfactant from 54.1 ± 1.7 mN m^{-1} (control; mean ± S.E.M.) to 49.2 ± 2.1 mN m^{-1} (surfactant; $P < 0.04$). Compared with control, P_O increased significantly ($P < 0.04$; paired t test, $n = 9$) from 6.2 ± 0.9 to 9.6 ± 1.2 cmH_2O with saline, and decreased significantly ($P < 0.05$, $n = 16$) from 6.6 ± 0.4 to 5.5 ± 0.6 cmH_2O with surfactant instillation. Findings tended to be similar for P_C. Change in both P_O and P_C showed a strong positive correlation with the change in γ of UAL (both $r > 0.70$, $P < 0.001$). In conclusion, the patency of the upper airway in rabbits is partially influenced by the γ of UAL. These findings suggest a role for UAL surface properties in the pathophysiology of OSA.

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Corresponding author J. P. Kirkness: Ludwig Engel Centre for Respiratory Research, Westmead Hospital, Westmead, New South Wales, Australia 2145. Email: jason_kirkness@wmi.usyd.edu.au

The obstructive sleep apnoea syndrome (OSA) is characterised by repetitive closure and re-opening of the upper airway during sleep. A widely accepted analysis of the control of upper airway patency is based on the concept that upper airway lumenal size will be dependent on the balance of forces acting across the upper airway walls (Remmers *et al.* 1978). While the role played by intraluminal pressure and the action of upper airway dilator muscles in determining this balance of forces has been extensively studied, little attention has been paid to other forces that may be in operation.

In 1980 Wilson and colleagues (Wilson *et al.* 1980) reported postmortem studies in infants demonstrating that the intraluminal pressure required to re-open a closed upper airway (P_O) was greater than the intraluminal pressure present during closure of the same airway (P_C). This difference between P_O and P_C was ascribed to the force required to overcome 'adherence' between the walls of the closed airway. These findings suggested that surface effects due to the liquid lining the upper airway (UAL) exert an influence on upper airway patency. Since these first observations there have been few studies that have addressed this concept. Olson & Strohl (1988*a*) demonstrated that stimulation of upper airway secretions in rabbits made the collapsed upper airway more difficult to re-open (i.e. increased P_O). This effect was ascribed to 'stickiness' of the induced upper airway secretions. In dogs, instillation into the upper airway of substances thought to have surface tension-lowering properties was associated with a reduction in airflow resistance (Widdicombe & Davies, 1988), decreased the degree of genioglossus muscle recruitment required to re-open the closed upper airway (Miki *et al.* 1992), and also decreased both P_O and P_C (Crawford *et al.* 1996).

In humans, studies are even more limited. Hoffstein *et al.* (1987) demonstrated reduced snoring in sleeping subjects after instillation of a 'long acting tissue lubricant' into the upper airway. More recently, Jokic *et al.* (1998) found that application of a topical lubricant consistently reduced the severity of OSA. These findings imply a pathogenetic role for UAL surface-related forces in OSA, and a potential role for therapeutic modulation of these forces in the treatment of OSA. An important contribution was made by van der Touw *et al.* (1997) who used fluoroscopy to study the patency of the upper airway in normal human subjects before and after instillation of a known exogenous surfactant (Exosurf Neonatal) into the upper airway. These studies demonstrated an ~7 cmH_2O reduction in P_C and ~19 cmH_2O reduction in P_O with surfactant but not with a saline control. Moreover, post-surfactant pharyngeal diameters were increased, relative to control, over most intraluminal pressures studied.

All the above studies suggest that forces due to the UAL play a measurable role in the determination of upper airway patency. However, this area of study is characterised by confusion regarding the nature of these forces and the lack of any direct measurements of the forces themselves. In addition, potential for the confounding effects of upper airway dilator muscle recruitment (Hoffstein *et al.* 1987; Van der Touw *et al.* 1997) and poor characterisation of the substances added to the upper airway (Widdicombe & Davies, 1988) limit the interpretation of some studies.

Recently, we described a method for measuring the surface tension (γ) of small volume (~0.2 μl) liquid samples and applied this method to the measurement of γ of saliva (Kirkness *et al.* 2000). This method assesses the force required to separate two curved surfaces bridged by a droplet of the liquid under examination. In the present study, we apply this approach to the assessment of the γ of UAL and its relationship to upper airway patency. We performed our studies in an anaesthetised animal model where upper airway muscle recruitment could be controlled and the direct effects of alteration of γ of UAL studied.

METHODS

Animals

Studies were performed in 25 adult male New Zealand White rabbits (3–4 kg). The protocol was approved by the Western Sydney Area Health Service Animal Ethics Committee.

Anaesthesia

Induction of anaesthesia was achieved via an intramuscular injection of ketamine (35 mg kg^{-1}) and xylazine (5 mg kg^{-1}). Surgical preparation was performed whilst rabbits were anaesthetised with either ketamine (40 mg h^{-1})/xylazine (12 mg h^{-1}) delivered intravenously, or halothane (1–2 %) via inhalation. Following instrumentation, anaesthesia during the data-gathering phase of the protocol was maintained with intravenous sodium pentobarbitone (24 mg h^{-1}). Animals were killed at completion of the study, using an overdose of intravenous sodium pentobarbitone.

Surgery

Rabbits were studied in the supine posture. A tracheostomy was performed between the third and the fourth tracheal cartilage rings. Both the proximal and distal tracheal segments were cannulated. The caudal tracheal stump was connected to a pressure cycled ventilator (BT200, Bourns Life Systems, Riverside, CA, USA; 4–5 cmH_2O maximum pressure; inspiratory:expiratory ratio 1:1.5; 50 cycles min^{-1}; plus supplemental oxygen). The oesophagus was isolated and tied off at the level of the larynx.

Experimental set up

The mouth was taped shut and a mask was placed over the snout (sealed with petroleum jelly). The system was leak free to a positive air pressure of ~15 cmH_2O over 30 s. A 5 ml syringe was connected to the caudal end of the cranial tracheal stump and then used to systematically inflate and deflate the isolated upper airway. The volume of gas injected into the upper airway (ΔV) was measured using a linear slide potentiometer attached to the plunger of the syringe. Separate pressure transducers (Celesco ±200 cmH_2O, IDM Instruments, Dandenong, Australia) were used to monitor the pressure inside the mask (P_M) and in the cranial tracheal stump (P_{UA}). Data were digitised (MacLab 16 s,

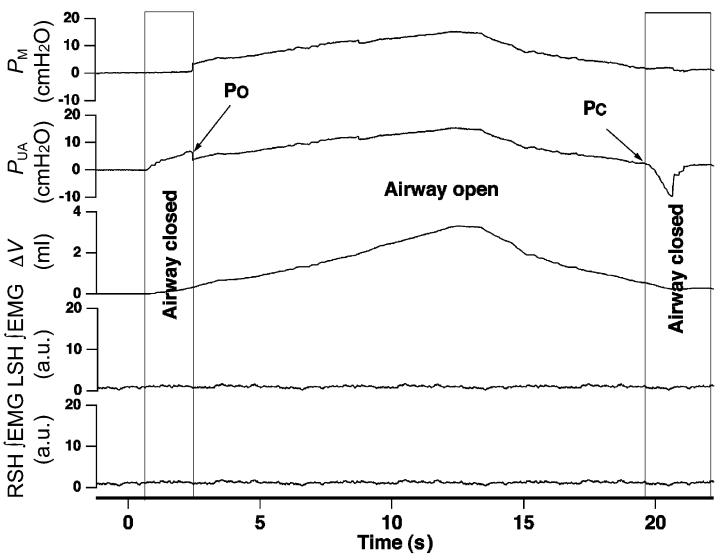

Figure 1. Raw data recording opening and closing pressures

Raw data recording showing pressure in the face mask (P_M), pressure recorded at the caudal end of the upper airway (P_{UA}), and change in upper airway volume (ΔV). As air is added to the upper airway P_{UA} increases immediately, whereas P_M does not change (airway closed) until a critical P_{UA} is reached i.e. the upper airway opening pressure (P_O). During the withdrawal of air from the upper airway a P_{UA} is reached where P_M no longer changes in parallel with P_{UA}, i.e. the upper airway closing pressure (P_C). Phasic ∫EMG activity was absent for both left (LSH) and right sternohyoid (RSH) muscles throughout the measurement.

ADInstruments, Sydney, Australia) and stored on a Macintosh computer for later analysis.

Electromyograms

Fine wire bipolar electrodes were positioned under direct vision in both the left and right sternohyoid (SH) muscle. The raw electromyographic (EMG) signals were filtered (80 Hz to 1 kHz), amplified, rectified and passed through a leaky integrator with a time constant of 100 ms to produce moving time average electromyograms (Neotrace NT 1900, Neomedix Systems, Sydney, Australia). The raw EMG signals were also connected to a speaker box. Correct placement of the wires was confirmed by: (1) the appropriate neck muscle contraction produced in response to direct electrical stimulation of the muscle; and (2) auditory confirmation of increased inspiratory motor unit activity during hypercarbic stimulation of ventilation. The integrated SH EMG was then monitored continuously throughout the protocol.

Surface tension measurements

The UAL was sampled by advancing polyethylene tubing (i.d. 0.5 mm; o.d. 0.8 mm) into the pharynx via the cranial tracheal stump and aspirating with a 1 ml syringe (7000.5N, Terumo Medical Corporation, Elkerton, USA), thus drawing a small quantity (~0.2 μl) of UAL into the tubing. Samples were transferred to the surface force measurement device and the γ of UAL was then measured via the 'pull-off' force technique (Kirkness *et al.* 2000). In addition, the γ of a saliva sample obtained from the oral cavity immediately after anaesthesia induction was obtained as a pre-surgery control value.

Exogenous surfactant

The γ of UAL was altered by instilling an exogenous surfactant into the upper airway. Exosurf Neonatal (Exosurf Neonatal; GSK, Greenville, NC, USA) is stored under vacuum as a sterile white lyophilised powder in vials. Each vial contains 108 mg colfosceril palmitate formulated with 12 mg cetyl alcohol, 8 mg tyloxapol and 47 mg NaCl. When reconstituted with 8 ml of sterile water, Exosurf Neonatal suspension contains 13.5 mg ml^{-1} colfosceril palmitate, 1.5 mg ml^{-1} cetyl alcohol and 1 mg ml^{-1} tyloxapol in 0.1 M NaCl, and has an osmolarity of 185 mosmol l^{-1}. The γ of Exosurf Neonatal has been reported to be between 38 and 44 mN m^{-1} (Schurch, 1993; Amirkhanian & Merritt, 1995).

Protocol

Following initial sampling of UAL and measurement of γ, 3–5 ml of air were injected into the upper airway at a rate of 0.2–1.0 ml s^{-1} until both P_M and P_{UA} reached 10–15 cmH_2O. Air was than slowly withdrawn from the upper airway until P_M no longer changed while P_{UA} continued to fall. This quasi-static cycle was repeated 5–7 times per run with 2–6 runs being performed for each condition in each rabbit. Measurement of the γ of UAL was then repeated.

Following collection of control data, 0.5 ml of either 0.9 % saline (saline; $n = 9$) or an exogenous surfactant (surfactant; $n = 16$; Exosurf Neonatal) was instilled into the pharyngeal airway, via a multi-holed catheter advanced through the tracheostomy and the protocol was repeated.

Data analysis

The P_O and P_C were measured using a technique similar to that described by Olson & Strohl, (1988*b*). As air was injected into the upper airway increasing P_{UA}, P_M did not immediately change (i.e. the upper airway was spontaneously closed in all rabbits). The value of P_{UA} when P_M began to increase was defined as P_O (Fig. 1). During withdrawal of gas from the upper airway, the value of P_{UA} at the point where P_M ceased to change was defined as P_C. For each inflation–deflation cycle, change in upper airway volume (ΔV) was plotted against P_{UA} generating partial upper airway pressure–volume relationships (from $P_{UA} = P_C$ to P_{UA} = 10–15 cmH_2O). Over most of the range of ΔV studied, these relationships were approximately linear, but hysteresis of the upper airway pressure–volume relationship was evident for all such measurements. Upper airway wall compliance was calculated from the slope of separate linear regression lines fitted to the linear portion of both the inflation (C_{UAI}) and deflation (C_{UAD}) limbs of the quasi-static pressure–volume curves (Fig. 2). The P_{UA} at a ΔV of 2 ml (Fig. 2) was measured for both the inflation (P_{IR}) and deflation (P_{DR}) regression lines. Hysteresis (H_{UA}) was measured as $P_{IR} - P_{DR}$.

Individual upper airway mechanics measurements were averaged to obtain mean values for each run. Run values were then averaged to obtain individual rabbit data for each condition. Individual rabbit values were then pooled to obtain group mean data. The γ of UAL for each condition was determined as the average of the values obtained before and after each set of upper airway mechanics measurements. Data were expressed as means ± S.E.M. Values for γ of UAL obtained before and after upper airway mechanics measurements were compared using Student's paired *t* test. Repeated measures analysis of variance (ANOVA) was used to assess the effect of saline or surfactant administration on γ of UAL, P_O, P_C, $P_O - P_C$, P_{IR}, P_{DR} and H_{UA}. For the measured variables where there was a statistically significant difference between the change observed within rabbits instilled with surfactant and that

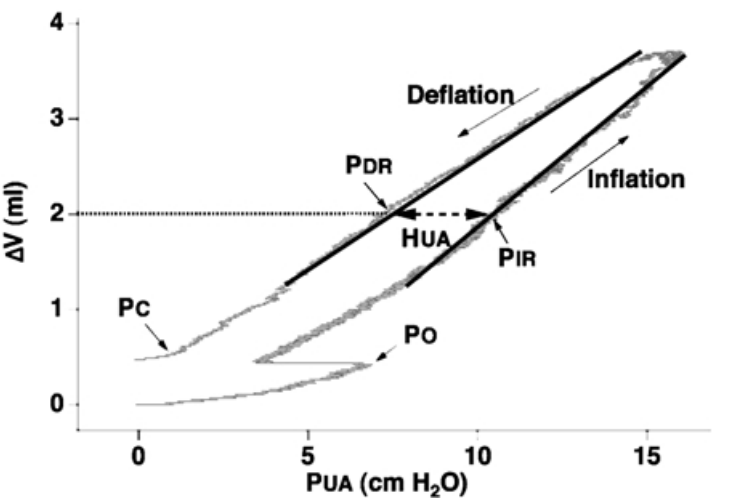

Figure 2. Upper airway wall compliance and recoil pressures

Representative upper airway pressure (P_{UA}) volume (ΔV) recording during control conditions in one rabbit. Upper airway wall compliance was calculated as the slope of the linear regression lines (continuous lines) fitted to the inflation and deflation limbs of the pressure–volume relationship over the same volume range. P_C closing pressure, P_O opening pressure, P_{DR} deflation recoil pressure, P_{IR} corresponding inflation recoil pressure. Hysteresis (H_{UA}; dashed line) was calculated as $P_{IR} - P_{DR}$.

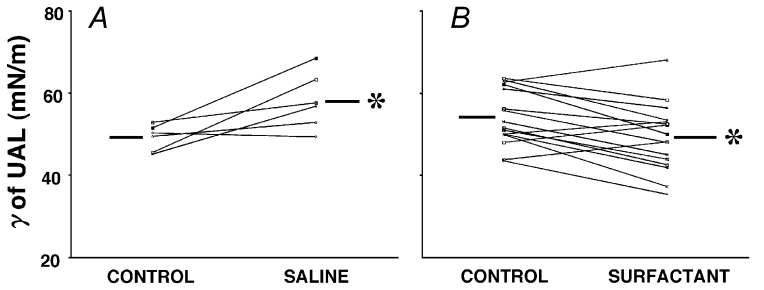

Figure 3. Effect of exogenous surfactant and saline on γ of UAL

Individual data for γ of UAL under control *vs.* saline (*A*) and control *vs.* surfactant (*B*) conditions. Note that increases in γ of UAL occurred in the majority of rabbits with saline, while decreases occurred in most rabbits with surfactant. Different lines represent individual rabbits. * $P < 0.05$ *vs.* control. Bars represent group mean values.

observed in those instilled with saline, the groups were analysed separately. Individual relationships between the change in γ of UAL associated with saline and surfactant instillation and the corresponding change in P_O, P_C, C_{UAI}, C_{UAD}, H_{UA} and $P_O - P_C$ were all tested using linear regression analysis. $P < 0.05$ was considered significant.

RESULTS

EMG data

No phasic EMG activity of the SH muscles was detected throughout the study (see Fig. 1).

Surface tension of upper airway lining liquid

Under control conditions, measurements of γ of UAL were obtained in 22 rabbits, six prior to saline and 16 prior to surfactant. There was no significant difference ($P > 0.2$) between the γ of UAL before and after the measurement of upper airway mechanics. During control, the γ of UAL ranged from 43.6 to 63.6 mN m^{-1} with a mean value of 54.2 ± 1.3 mN m^{-1} ($n = 22$). Following saline, γ of UAL increased by > 3.7 mN m^{-1} in four rabbits but was unchanged in the remaining two rabbits. For the group, γ of UAL increased from 52.1 ± 1.9 to 58.1 ± 2.1 mN m^{-1} ($n = 6$; $P < 0.04$; Fig. 3). Following surfactant, γ of UAL decreased by > 3.6 mN m^{-1} in 12 rabbits but increased by > 3.0 mN m^{-1} in the remaining four rabbits. For the group, γ of UAL decreased from 54.1 ± 1.7 to 49.2 ± 2.1 mN m^{-1} ($n = 16$; $P < 0.005$; Fig. 3).

For all rabbits the pooled data ($n = 19$) for γ of the pre-surgery saliva samples (54.4 ± 1.1 mN m^{-1}) was not significantly different from that of all control UAL samples obtained from the posterior pharynx (54.3 ± 1.5 mN m^{-1}; $P = 0.95$). These saliva data for seven animals have been reported previously (Kirkness *et al.* 2000).

Upper airway opening pressure

Following saline, P_O increased by > 1.8 cmH_2O in six of nine rabbits but was unchanged in the remaining three rabbits (Fig. 4*A* and *C*). Following surfactant, P_O decreased by >1.5 cmH_2O in nine of 16 rabbits, increased by

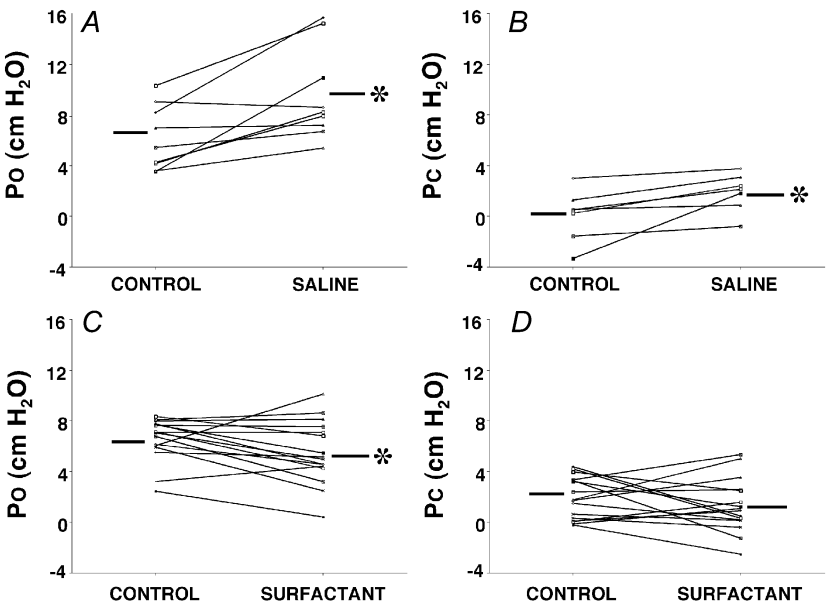

Figure 4. Effect of exogenous surfactant and saline on P_O and P_C

Individual data for P_O (*A* and *C*) and P_C (*B* and *D*) under control, saline and surfactant conditions. Note that increases in both P_O and P_C occurred in the majority of rabbits with saline, while decreases occurred in most rabbits with surfactant. This was a significant change for all conditions except for P_C with surfactant. Different lines represent individual rabbits. * $P < 0.05$ *vs.* control. Bars represent group mean data.

Table 1. Upper airway wall compliance values

	Control ($ml\ cmH_2O^{-1}$)	Saline ($ml\ cmH_2O^{-1}$)	Control ($ml\ cmH_2O^{-1}$)	Surfactant ($ml\ cmH_2O^{-1}$)
C_{UAI}	0.38 ± 0.02	0.36 ± 0.02	0.29 ± 0.02	0.29 ± 0.03
C_{UAD}	0.32 ± 0.02 *	0.28 ± 0.01 †	0.25 ± 0.02 *	0.24 ± 0.02 ‡

Upper airway inflation (C_{UAI}) and deflation (C_{UAD}) compliance values (means ± S.E.M.).
For C_{UAI} *vs.* C_{UAD}: * $P < 0.003$, † $P < 0.03$, ‡ $P < 0.001$.

> 0.5 cmH_2O in three and remained unchanged in four rabbits. For the group, P_O increased significantly from 6.2 ± 0.9 cmH_2O (control) to 9.6 ± 1.2 cmH_2O ($P < 0.04$) with saline, and decreased significantly from 6.6 ± 0.4 to 5.5 ± 0.6 cmH_2O ($P = 0.05$) with surfactant (Fig. 4). There was no difference between the control values for the two groups ($P = 0.6$).

Upper airway closing pressure

Technically acceptable measurements of P_C were obtained in seven rabbits for saline and in all 16 rabbits for surfactant. Following saline, P_C increased by > 0.8 cmH_2O in five rabbits, but was unchanged in the remainder (Fig. 4*B* and *D*). Following surfactant, P_C decreased by > 0.7 cmH_2O in eight rabbits, increased by > 0.8 cmH_2O in six and was unchanged in the two remaining rabbits. For the group, while P_C increased significantly from 0.1 ± 0.7 to 1.9 ± 0.6 cmH_2O with saline ($P < 0.03$) there was no significant change with surfactant (1.9 ± 0.8 *versus* 1.3 ± 0.5 cmH_2O, $P = 0.3$). Saline control values were significantly lower than surfactant control values ($P < 0.03$) primarily because of negative values obtained in two rabbits.

$P_O - P_C$ and H_{UA}

In all rabbits in which both P_O and P_C measurements were obtained ($n = 23$), P_O was greater than P_C under all conditions. There was a tendency for the $P_O - P_C$ group mean values to increase with saline (5.2 ± 0.6 *versus* 6.0 ± 0.7 cmH_2O, $n = 7$) and to decrease with surfactant (4.8 ± 0.3 *versus* 4.3 ± 0.3 cmH_2O, $n = 16$); however, neither of these changes achieved significance (both $P = 0.13$).

For P_{IR}, P_{DR} and H_{UA} there were no statistically significant differences between the within-rabbit changes for saline and surfactant (all $P > 0.17$), consequently, an overall test for change in the combined groups was performed. For P_{IR}, saline and surfactant control values were 8.1 ± 0.7 and 9.9 ± 0.5 cmH_2O, respectively. For P_{DR} these values were 5.8 ± 0.8 and 7.2 ± 0.6 cmH_2O, respectively. There was no significant effect of saline or surfactant on P_{IR} or P_{DR} (all $P > 0.3$). Similarly, for H_{UA}, saline and surfactant control values were not significantly different (2.3 ± 0.2 *versus* 2.7 ± 0 cmH_2O) and there was no change from control for either saline (2.5 ± 0.1 cmH_2O) or surfactant (2.4 ± 0.2 cmH_2O; all $P = 0.8$).

Upper airway wall compliance

Upper airway wall compliance data were obtained in three rabbits for saline and in 14 rabbits for surfactant. While C_{UAI} was significantly greater than C_{UAD} for control ($P < 0.003$; paired t test), and for both saline ($P < 0.03$) and surfactant ($P < 0.001$), neither saline nor surfactant was associated with a change in the group-mean values for C_{UAI} or C_{UAD} ($P > 0.2$; Table 1).

Relationship between γ of UAL and upper airway patency

When all data were pooled, changes in P_O and P_C (both $r > 0.7$, $P < 0.001$; Fig. 5) and change in $P_O - P_C$ ($r = 0.41$,

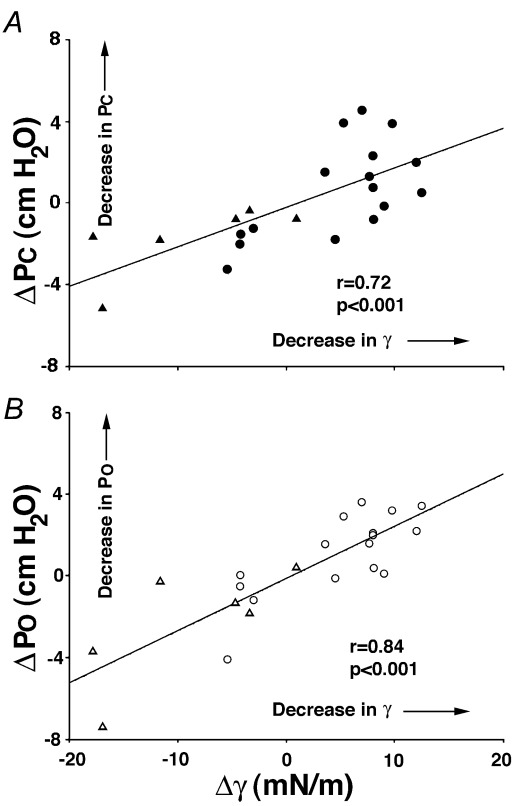

Figure 5. Influence of changing γ on upper airway mechanics

Change (control minus saline (triangles) or surfactant (circles) P_C (in ΔP_C; *A*, filled symbols) and P_O (in ΔP_O; *B*, open symbols) for each rabbit plotted against change in γ of UAL ($\Delta\gamma$;control minus saline or surfactant). Linear regression lines are shown. Note the strong positive correlations between $\Delta\gamma$ and both ΔP_C and ΔP_O.

$P = 0.05$), but not change in H_{UA} ($P > 0.3$), were positively correlated with change in γ of UAL. However, a negative correlation was found between change in C_{UAD} (but not change in C_{UAI}; $P > 0.1$) and change in γ of UAL ($r = 0.6$, $P < 0.005$). There was no significant relationship between change in P_{IR} and P_{DR} and change in γ of UAL (both $r < 0.26$, $P > 0.1$).

DISCUSSION

This study has established, for the first time, a quantitative relationship between the γ of UAL and mechanical factors influencing upper airway patency. In particular, we have demonstrated that in anaesthetised rabbits there are significant correlations between change in γ of UAL and changes in P_O, P_C, C_{UAD} and $P_O - P_C$, but not P_{IR}, P_{DR} or H_{UA}. When γ of UAL was increased (by instillation of normal saline into the upper airway) the airway both closed and re-opened at a more positive intraluminal pressure than under control conditions. Whereas, when the γ of UAL was reduced (by the instillation of an exogenous surfactant) the airway closed and re-opened at reduced positive intraluminal pressures. These findings support the hypothesis that γ of UAL contributes a force acting on the upper airway wall that hinders airway opening but is modifiable through the instillation of surface active agents into the upper airway lumen.

Critique of methods

The rabbit upper airway model has been employed extensively in previous studies of upper airway mechanics including those of the recruitment and mechanical effects of upper airway dilator muscles (Rothstein *et al.* 1983; Olson *et al.* 1989; Woodall *et al.* 1989). Recruitment of upper airway dilator muscles such as the genioglossus and sternohyoid muscles constitutes a potential confounding effect in studies examining the influence of γ of UAL on upper airway patency because these muscles are strongly recruited by negative upper airway pressure and have important effects on upper airway lumen size and collapsibility (Mathew *et al.* 1982*a*,*b*; Rothstein *et al.* 1983). In the present study we deliberately suppressed upper airway muscle activity by using a protocol featuring isolation of the upper airway, mechanical ventilation and deep barbiturate anaesthesia. Lack of upper airway dilator muscle recruitment during measurement of P_O and P_C was confirmed by monitoring SH muscle EMG activity.

UAL samples were obtained by advancing a catheter into the pharynx via the cranial tracheal segment. It is possible that this sampling method may have failed to obtain a representative sample of UAL. Similarly, exogenous surfactant and saline were introduced into the upper airway by a catheter advanced blindly into the pharynx. Non-uniform distribution of these agents may be responsible for the failure to lower the γ of UAL with exogenous surfactant in some rabbits. This failure to change γ of UAL uniformly in all rabbits impacted on our ability to detect an effect of surfactant instillation on P_C. Compliance, P_{IR}, P_{DR} and H_{UA} values were obtained from the linear portions of the pressure–volume relationships. This will have influenced the absolute values obtained since the entire pressure–volume relationship was not examined. However, these values represent the elastic properties of the airway wall when the airway is patent and were used to assess effects of changing γ of UAL when the distance between mucosal surfaces was relatively large.

A strength of the present study is the direct measurement of γ of UAL. All previous studies on this topic have assumed that the addition of exogenous surfactant to the upper airway changes upper airway surface properties but have made no measurements to confirm this assumption (Widdicombe & Davies, 1988; Miki *et al.* 1992; Crawford *et al.* 1996; Van der Touw *et al.* 1997; Jokic *et al.* 1998). In the present study, measurement of γ of UAL permitted the relationship between γ of UAL and upper airway mechanical properties to be examined directly.

Surface tension of UAL

The values for γ measured in the present study are the first measurements reported in the literature for UAL. At ~52 mN m^{-1} the γ of rabbit UAL is substantially less than that for water (71.2 mN m^{-1}; Lide, 2001) reflecting the presence of endogenous surfactants in rabbit UAL. While UAL has not been previously studied, there have been a number of previous studies examining the γ of saliva (Braddock *et al.* 1970; Glantz, 1970). Saliva is 95% water but contains small concentrations of phospholipids with surfactant properties (Demmers & Belting, 1967; Vassilakos *et al.* 1992). There are no studies that report γ for rabbit saliva, although reported values for the γ of human saliva range from 53.1 to 57.0 mN m^{-1} (Braddock *et al.* 1970; Glantz, 1970). In the current study, the γ of UAL was not different from that of the pre-surgery control saliva sample obtained from the oral cavity and was in the same range as that reported in the literature for human saliva. It appears that at least in regard to its surface force properties, the UAL of the pharynx is similar to saliva.

Instillation of saline into the pharynx was associated with an increase in the γ of UAL. This may be due to the following: (1) saline may lead to an increase in the secretion of glycopolysaccharides (Anderson *et al.* 1997); (2) saline may increase the re-absorption of surface active particles across the epithelial lining (Rahmoune & Shephard, 1994); or (3) isotonic saline in the upper airway may replace surface active substances, already in the UAL, with a high γ liquid (Hida & Hildebrandt, 1984). The lower γ of UAL after instillation of surfactant is attributed to exogenous surfactant adhering to the mucosal surface decreasing the free energy at the surface of the UAL (Scarpelli *et al.* 1992).

Balance of forces model

Upper airway patency is determined by the net balance of forces operating across the upper airway walls (Remmers *et al.* 1978). Upper airway collapsing forces have previously been attributed to intra-luminal negative pressures (Mathew *et al.* 1982*a*, 1984; Harms *et al.* 1996; Eastwood *et al.* 1998), upper airway constrictor muscle activity (Kuna & Smickley, 1997; Kuna & Vanoye, 1997), and compressive pressures exerted by the surrounding tissues (Winter *et al.* 1996, 1997). These collapsing forces are opposed by the intrinsic elastic properties of the airway wall and, most importantly, by upper airway dilator muscle activity (Strohl *et al.* 1987; Wiegand & Latz, 1991; van Lunteren & Manubay, 1992; Bishara *et al.* 1995).

The present study has now demonstrated that the γ of UAL is an additional property that influences the force necessary to open the upper airway. This may be particularly important when surfaces are apposed (i.e. airway closed) or in regions where the mucosa may fold forming tightly curved surfaces. A characteristic of the normal human upper airway is the presence of mucosal folds and this is accentuated in the case of OSA patients (Kuna *et al.* 1988). Surface forces may be operative in these folds keeping the mucosal surfaces in contact and contributing to both the thickening of the lateral pharyngeal walls and the elliptical cross-sectional shape of the pharyngeal airway characteristic of patients with OSA (Schwab *et al.* 1995).

Surface forces

The important surface-related forces in a system such as the upper airway mucosal surface are, under normal physiological conditions, related to the presence of fluid lining the mucosa. Both the γ and the viscosity of the upper airway fluid may play a role in these forces, and often a combination of the two will be important in considering the influence of surface forces on upper airway patency.

Liquid-coated surfaces will adhere to each other, and in the case of two ideally flat surfaces the force holding them together, or the contact adhesion, depends only on the γ of the liquid and the separation between the surfaces (d). If one postulates a continuous film that wets both surfaces, the pressure ΔP holding the surfaces together is $\Delta P = 2\gamma/d$. The smaller the surface separation and the larger the γ, the greater the force holding the surfaces together. As d may be a micron or less for smooth surfaces, the pressure can easily reach several atmospheres! Adhesion between liquid-coated surfaces is, thus, due to the γ of the liquid, which in turn is ultimately due to the cohesive forces between the molecules of the liquid. Reducing the γ by, for example, the addition of exogenous surfactant will reduce the adhesion.

However, the force required to separate the surfaces depends very much on the exact manner in which the surfaces are separated. A simple analogy may be made with two microscope glass slides between which a drop of water is placed. The water will form a very thin film between the two slides, and quite some force will be required to separate the two slides by pulling in a direction normal to the surfaces. In contrast, it is easy to slide the surfaces against one another and separate them by letting one surface slide completely away from the other. If instead of glass slides one has two flexible sheets of plastic, the sheets may be easily separated by peeling them away from one another. Furthermore, because the act of opening is a dynamic process, non-equilibrium processes related to the viscosity and possible visco-elasticity (stickiness) of the fluid may also come into play. These will act to retard the opening process (imagine a film of sticky honey between the surfaces) and the force will need to be applied for longer even to slide or peel the surfaces apart.

In the upper airway, the opposing tissues of the collapsed passage are obviously far from ideally smooth and flat, but because the tissues are deformable very intimate contact may well be achieved. Separation in the presence of a fluid film hence presents us with essentially the same overall problem to consider as in the case of two flexible sheets of plastic – the force required to effect separation of the tissue walls will very much depend on details of the opening process. In the upper airway, of course, the magnitude of the required force is subject to further complications such as folds of the airway being kept locally closed by pockets of mucosal fluid, regions of entrapped air in the closed airway, etc.

Nevertheless, it is clear that reducing the γ of the liquid lining the upper airway can only reduce the force required to separate the opposing surfaces, provided that factors such as viscosity are not greatly affected. It should here be noted that surfactants will significantly lower the γ of water at concentrations far below those that influence the viscosity.

Upper airway opening and closing pressures

In anaesthetised, tracheostomised, mechanically ventilated, supine rabbits, P_C was approximately equal to atmospheric pressure. When saline was added to the upper airway, P_C increased to ~2 cmH_2O (i.e. the airway was closed at a more positive intraluminal pressure). This finding suggests that application of saline to the upper airway is associated with an additional collapsing force of ~2 cmH_2O. When exogenous surfactant was added to the upper airway there was no group-mean change in P_C. This finding, however, appears to be related to the fact that instillation of surfactant failed to lower the γ of UAL in some rabbits. Indeed, a strong positive correlation between the change in P_C and the change in γ of UAL was identified when the saline and surfactant data were pooled.

Under control conditions a positive intraluminal pressure of ~6 cmH_2O was required to open the airway. Instillation

of saline into the upper airway was associated with an ~3 cmH_2O increase in P_O, while exogenous surfactant led to an ~1 cmH_2O decrease in P_O. In addition, in the present study there was a strong positive correlation between the change in P_O and the change in γ of UAL. These findings suggest that the γ of UAL exerts a measurable collapsing force on the upper airway that can be modified by the instillation of surface active agents into the upper airway.

Upper airway wall compliance

Upper airway wall compliance values of ~0.2–0.3 ml cmH_2O^{-1} were obtained in the present study, a value about double that reported for rabbits by Olson and co-workers (Olson *et al.* 1989). The more compliant upper airway demonstrated in the present study is likely to be related to the deliberate suppression of upper airway dilator muscle activity. While the airway was less compliant during deflation than during inflation, C_{UAI} was unaffected by change in γ of UAL. However, change in C_{UAD} was negatively correlated with change in γ of UAL. Thus, during deflation of the upper airway a decrease in γ of UAL was associated with an increase in wall compliance, reflecting a reduction in the collapsing force exerted by γ of UAL on the upper airway walls.

$P_O - P_C$ and H_{UA}

Previous studies have consistently demonstrated a difference between upper airway P_O and P_C in both animal (Crawford *et al.* 1996) and human studies (Wilson *et al.* 1980; Van der Touw *et al.* 1997). In the present study change in $P_O - P_C$ was positively correlated with change in γ of UAL. Thus, as γ of UAL fell the difference between P_O and P_C was reduced. However, this relationship appeared to only explain 17 % of the variance indicating that while γ of UAL does contribute to $P_O - P_C$, other factors may have a much greater influence. Thus, over the range of γ studied, the contribution of γ of UAL to the adherence of upper airway walls only explains a portion of the effect. Other factors, such as non-equilibrium surface forces (e.g. viscosity) and inertial factors associated with the airway walls and surrounding tissues may be more important.

While we were able to demonstrate a relationship between upper airway wall recoil pressure at opening and closing and γ of UAL this was not the case when the airway was already open. Thus, P_{IR}, P_{DR} and H_{UA} were not influenced by the range of γ of UAL studied. This might be expected since the distances separating upper airway mucosal surfaces would be much larger than at the point of airway closure. Since H_{UA} was unaffected by γ of UAL it would seem that hysteresis of the upper airway wall pressure volume relationship (above P_C) is more related to the elastic properties of the surrounding tissues than the γ of UAL.

Conclusion

In the present study, following saline or surfactant instillation into the upper airway, the change in γ of UAL correlated strongly with the change in both P_O and P_C. The association of a change in P_O with a change in γ of UAL means that as the γ forces of the liquid in the upper airway are lowered the airway becomes easier to re-open. Similarly, the ability of the upper airway to remain open under the influence of a collapsing force is enhanced when the liquid lining the upper airway has lower γ forces. Thus, γ is potentially an important factor determining airway patency especially when airway walls are apposed or nearly apposed (i.e. the distances separating the mucosal surfaces are small). This is the first study to measure and examine γ of UAL in relation to upper airway wall mechanical properties. We conclude that patency of the upper airway is influenced by γ of UAL and that this relationship can be manipulated by instillation of surface active agents into the upper airway.

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