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Developmental Differences in Tracheal Cartilage Mechanics

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ABSTRACT. Length-tension characteristics were examined in tracheal rings excised from preterm lambs (*n* = 8) and adult sheep (*n* = 7) to determine developmental differences in the mechanical properties of tracheal cartilage. Tension was measured in both the complex (comprised of the pars membranacea and its cartilagenous insertion) and the cartilage alone (by severing the pars membranacea) as the ring was lengthened so as to increase the distance between the points of insertion. Analysis of regression lines show significantly greater slopes for both the complex and the cartilage in the adult group compared to the preterm group. These data reveal that age-related differences in tracheal cartilage mechanics parallel and may contribute to age-related differences previously determined in tracheal compliance. (*Pediatr Res* 26: 429–433, 1989)

Abbreviations

PM, pars membranacea
ACh, acetylcholine
CT, cartilage tension
TPT, total passive tension

In previous studies we have shown tracheae of preterm lambs to be extremely compliant structures (1, 2). We have also established the stiffness of tracheal smooth muscle (contained in the posterior wall) as an important determinant of tracheal compliance (3), and have noted developmental differences in tracheal smooth muscle passive tension generation (4).

Several authors (3, 5, 6) have suggested that tracheal cartilage also plays an important role in determining tracheal compressibility and inflatibility. Moreno *et al.* (5) softened rabbit airway cartilage with papain and demonstrated alterations in unstressed tracheal volume and tracheal compliance. In a related study using papain-treated rabbits, McCormack *et al.* (7) observed changes in pulmonary function indicative of increased airway collapsibility.

Developmental differences in the mechanical properties of tracheal cartilage have not previously been studied. Such differences may contribute to the observed developmental differences in tracheal compliance. To test this hypothesis we examined length-tension characteristics of tracheal ring segments from both premature and adult sheep. Mechanical properties of cartilage were calculated apart from those of the entire ring providing an index of cartilage stiffness in each group. These data were examined for possible age-related differences.

MATERIALS AND METHODS

Tracheal segments containing one cartilaginous ring were excised from premature lambs (*n* = 8; 118–120 d gestation) from a position approximately 20 mm above the carina. Three rings from each trachea were cut from this area and the experimental values from these three segments were averaged to represent one experiment. Each segment was cut and trimmed so that the width of the PM was equal to the width of the cartilaginous ring. The external fascia was removed from the ring. The segment was then mounted into a tissue bath using two ties so that the axial smooth muscle fibers within the PM were vertically oriented (Fig. 1). The lower tie attached at one point of insertion of the PM into the cartilage was attached rigidly to the bottom of the bath. The upper tie attached to the other point of insertion was attached via a gold chain to an isometric force transducer, and the ring remained in a fixed plane throughout the course of the length-tension measurements. The length of the PM was determined by measuring the distance between the points of PM insertion into the cartilage. The resting length was defined as that PM length when no external forces were applied to the tracheal ring. A 1.5-g wt was suspended from the upper tie and served to pull the cartilaginous tips more closely together and therefore reduce the length of the PM to below the resting length. The transducer was mounted to a micrometer so that the length of the PM could be varied. The mounted ring was bathed in Krebs-Ringer solution. Temperature was maintained at 37°C, and a gaseous mixture of 95% O_2/5% CO_2 was continuously bubbled through the bath to maintain a pH of 7.40 ± 0.03.

All transducers were connected to a Grass model 7 polygraph (Braintree, MA), and tensions were continuously recorded throughout the entire experiment on a strip chart recorder. After mounting, the ring was allowed to stabilize in the bath for 1 h. The PM was then stretched until a finite tension was recorded after stress relaxation had occurred. The smooth muscle of the PM was then contracted with 10^{-5} M ACh and peak active tension was achieved.The segment was then washed free of agonist, the PM was stretched an additional 1.0 mm, TPT was recorded, and the procedure was repeated at increasing lengths until peak active tension was determined. The length at which peak active tension occurred was designated Lo.

The PM was then returned to its initial length and washed free of agonist. To ensure that passive length-tension data were not influenced by residual smooth muscle tone, the procedure was repeated without ACh administration and active tension generation. Data obtained under these circumstances were identical to that obtained when the methods included ACh administration.

The PM was then cut so that the recorded tension solely reflected the contribution of the cartilaginous ring. The distance between the tied ends of cartilage was increased by 1.0-mm intervals and tension was recorded throughout the range of lengths established above.

Tracheal rings were also excised from adult sheep (*n* = 7) and prepared in a manner similar to that described above. Length-

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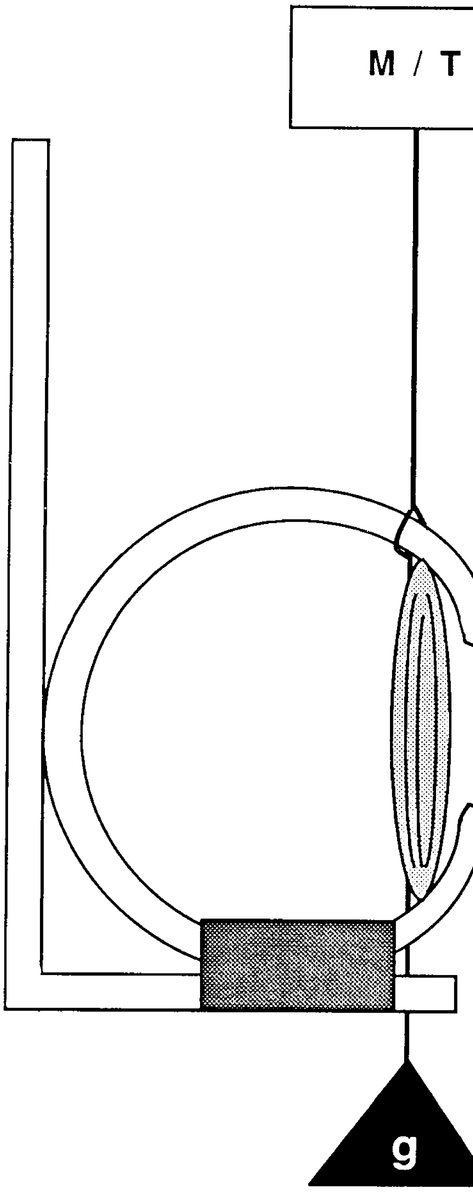

Fig. 1. Mounting of a tracheal ring in a tissue bath. The ring is tied at its base into a fixed vertical plane where tension is measured by a transducer (*T*); the length of the pars membranacea is altered by a micrometer (*M*). A weight is suspended from the upper tie in order to initially compress the ring and increase the range of measured lengths.

tension procedures on both the entire segment as well as the cartilage alone were repeated as described above.

Measurements of Lo were determined with a digital caliper when the ring was outside the bath. Polygraph recordings were checked repeatedly for baseline drift, and tension measurements were accurate up to 0.05 g.

Data reduction. For each experiment, the equations describing the lines TPT (g) *versus* PM length (mm), and CT (g) *versus* PM length (mm) were determined by linear regression. To normalize data for the purpose of a developmental comparison, raw data were transformed in the following manner. In both the preterm and adult groups, TPT and CT were expressed as a percent of the TPT at Lo (designated %TPTLo and %CTLo, respectively) and plotted as a function of length (expressed as percent of Lo). The equations describing the two lines formed by these points were determined by a first order regression. Differences in preterm *versus* adult values of the slopes of the %TPTLo and %CTLo lines were determined by linear regression analysis (BMDP Statistical Software, Berkeley, CA).

The mean tension exerted by cartilage when TPT = 0 (the force of cartilage on relaxed tracheae) was calculated as the arithmetic mean of individual ring values.

RESULTS

Typical length-tension relationships from premature and adult tracheal rings are presented in Figure 2. First order regression of data points produced lines that were linear in each group. Table 1 depicts the first order regressions of these lines for each experiment. The mean slopes of the lines in the adult group for both total passive tension and cartilage tension were approximately nine times greater than the corresponding mean values in the preterm group.

Total passive tension and CT values from each experiment were converted to a percentage of the TPT recorded at Lo and were plotted as a function of %Lo for both preterm (Fig. 3*A*) and adult (Fig. 3*B*) groups. In the preterm group, %TPTLo as a function of %Lo was represented by the first order regression $y = 4.49x - 304$ ($r = 0.77$); %CTLo *versus* %Lo by the line $y = 2.04x - 177$ ($r = 0.91$) (Fig. 3*A*). As might be expected, the %TPTLo exceeded the %CTLo except at very small lengths where the PM actually became compressed. The length of the PM in the relaxed ring had a mean value of 71.6%Lo, and the ring exerted a mean outward force of 0.34 ± 0.24 SD g.

In the adult group, %TPTLo as a function of %Lo was represented by the line $y = 8.79x - 760$ ($r = 0.86$); %CTLo *versus* %Lo by the line $y = 5.44x - 515$ ($r = 0.76$) (Fig. 3*B*). The measurable range for the adult ring was considerably smaller than that of the preterm ring. Force development at lengths more than 117% Lo or less than 80% Lo was sufficiently great as to

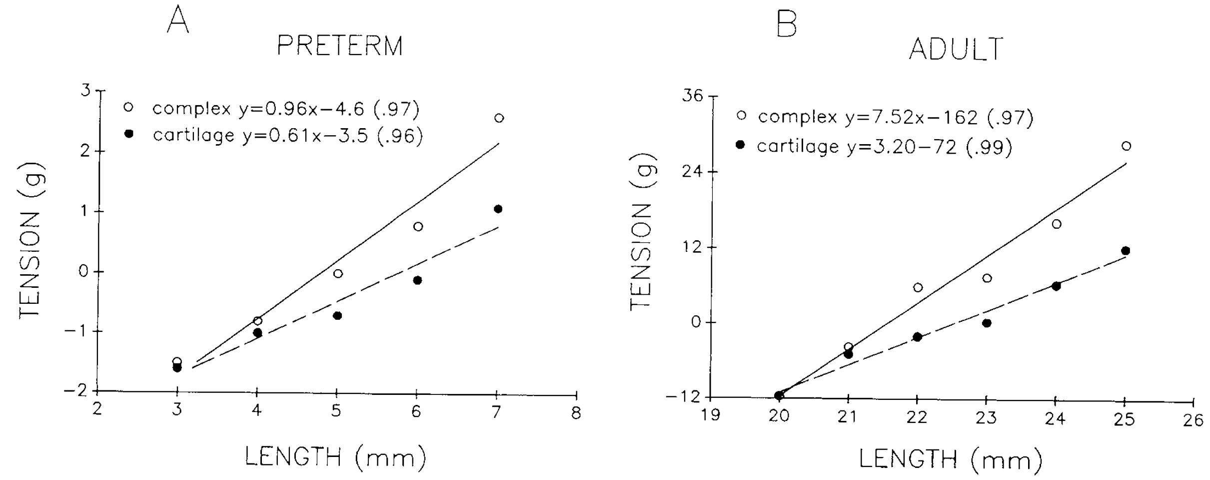

Fig. 2. Representative example of tension generation (complex: *open circles, solid lines*; cartilage: *filled circles, dashed lines*) as a function of pars membranacea length in a preterm (*A*) and adult (*B*) tracheal ring.

Table 1. *Equations of lines total passive tension (y_1) versus length (x) and cartilage tension (y_2) versus length (x) determined by first order regression**

Experiment	Lo (mm)	Regression (*r*)
Preterm		
1	6.0	$y_1 = 0.96x-4.6$ (0.97) $y_2 = 0.61x-3.5$ (0.96)
2	5.5	$y_1 = 0.82x-3.4$ (0.95) $y_2 = 0.39x-1.88$ (0.98)
3	6.0	$y_1 = 0.77x-3.2$ (0.92) $y_2 = 0.30x-1.6$ (0.95)
4	7.5	$y_1 = 0.37x-1.5$ (0.97) $y_2 = 0.31x-2.3$ (0.95)
5	8.0	$y_1 = 0.61x-3.5$ (0.95) $y_2 = 0.46x-3.3$ (0.97)
6	6.0	$y_1 = 0.77x-3.2$ (0.92) $y_2 = 0.18x-6.9$ (0.98)
7	7.0	$y_1 = 1.86x-8.8$ (0.88) $y_2 = 0.38x-2.5$ (0.91)
8	8.0	$y_1 = 0.49x-2.3$ (0.91) $y_2 = 0.24x-1.1$ (0.95)
Adult		
9	22.0	$y_1 = 4.75x-80$ (0.99) $y_2 = 3.03x-55$ (0.99)
10	24.0	$y_1 = 7.52x-162$ (0.97) $y_2 = 3.20x-72$ (0.99)
11	24.5	$y_1 = 7.51x-169$ (0.98) $y_2 = 4.42x-99$ (0.94)
12	25.0	$y_1 = 5.10x-115$ (0.90) $y_2 = 3.51x-86$ (0.99)
13	38.5	$y_1 = 4.97x-161$ (0.99) $y_2 = 1.32x-46$ (0.96)
14	36.5	$y_1 = 8.84x-241$ (0.99) $y_2 = 5.72x-62$ (0.99)
15	32.0	$y_1 = 3.74x-105$ (0.99) $y_2 = 2.18x-67$ (0.96)

* Data points used for regression determination fell within the range of approximately 40–120% of Lo.

exceed the accurate measuring capability of our transducer and produced data points that ascribed marked nonlinearity to the regression lines. The relaxed adult tracheal ring exhibited a PM length of 86.9%Lo with the cartilage exerting an outward force of 3.13 ± 1.33 SD g.

Normalized length-tension data were replotted to facilitate preterm/adult comparison for both %TPTLo (Fig. 4*A*) and %CTLo (Fig. 4*B*). Comparison of the regression line slopes for the complex shows a significantly ($p < 0.05$) lesser slope in the preterm group. In a similar manner, the slope of the regression lines for %CT are significantly ($p < 0.05$) different. Age-related differences in the slopes of these lines persist when the range of data points is more closely matched by eliminating data points <75%Lo (these points exist only in the preterm group and tend to skew the data). Our capacity to measure these points in the preterm group was attributed to the extreme compliance of the immature cartilage; attempts to reduce the PM length of the adult tracheal rings less than 75% required extremely heavy suspension weights that rendered accurate tension measurement within a fixed plane virtually impossible.

DISCUSSION

The results from our study suggest that tracheal cartilage of the premature lamb is extremely compliant compared to that of the adult sheep, as evidenced by differences in length-tension characteristics. These age-related differences appear to parallel developmental differences already observed in tracheal smooth muscle and tracheal mechanics (1–4). Age-related differences in tracheal compliance may therefore reflect an increase in stiffness that occurs with both components with maturation.

Airway compliance. High airway compliance has been suggested to be an important determinant of respiratory function (1–3, 8) and respiratory pathology (1, 9–11) in the immature lung. Specifically, high tracheal compliance in the premature lamb has been shown to alter pressure-flow relationships in both *in vitro (1, 3) and in vivo* (2) models, and may contribute to the high airway resistance and flow limitation observed in preterm infants (3). Furthermore, high compliance in both proximal and distal airways may predispose preterm infants to positive-pressure-induced damage such as volume deformation (1, 9, 10) tracheomegaly (11), or bronchopulmonary dysplasia (12–14). Histologic sections of conventionally ventilated lung from the 11-d preterm baboon demonstrate marked overdistension of the major conducting airways (13), although in a more chronic baboon model (14), these well ventilated but deformed areas of lung exist side by side with poorly ventilated but uninjured areas. These findings underscore the importance of airway mechanics in determining not only lung mechanics but ventilation homogeneity and gas exchange in the ventilated infant.

Tracheal smooth muscle tone and the mechanical properties of the PM have been shown to influence tracheal compliance in

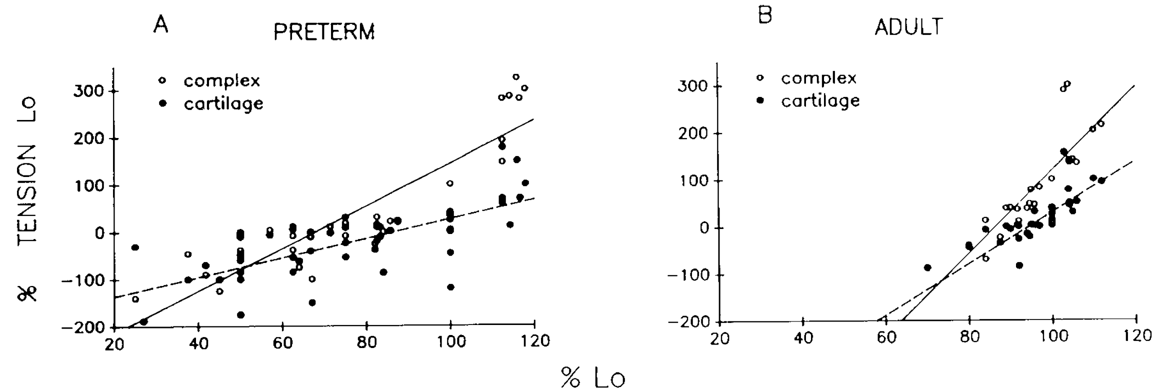

Fig. 3. Normalized complex, cartilage tension as a function of %Lo in premature (*A*) and adult (*B*) sheep. Total passive tension (complex, *open circles, solid lines*) and cartilage tension (*filled circles, dashed lines*) each represented as a percent of the total passive tension at Lo, is plotted as a function of length represented as a percent of the determined Lo. In both groups the slope of the length-tension lines for the complex were greater than that of the cartilage alone.

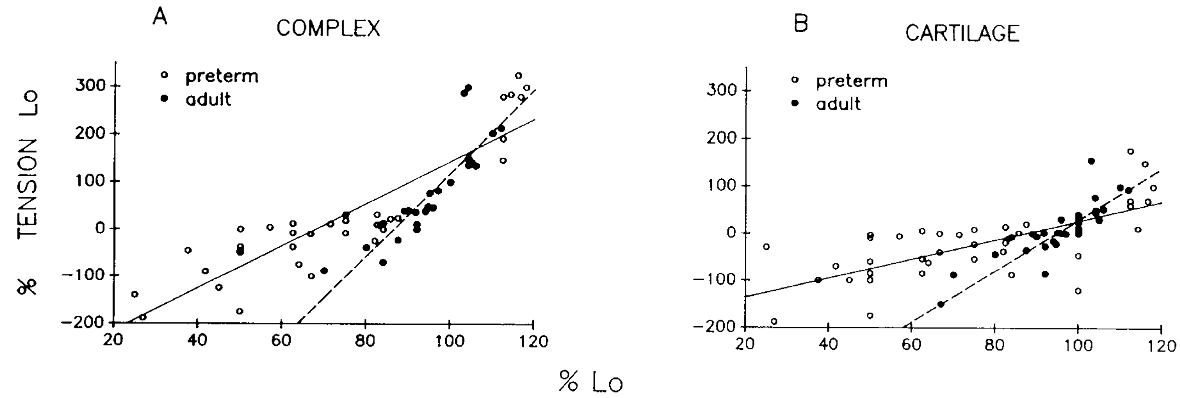

Fig. 4. Normalized complex (*A*) and cartilage (*B*) tension as a function of %Lo. In both components the slope of the length-tension line of the preterm group (*open circles, solid line*) was significantly ($p < 0.05$) less than that of the adult group (*closed circles, dashed line*).

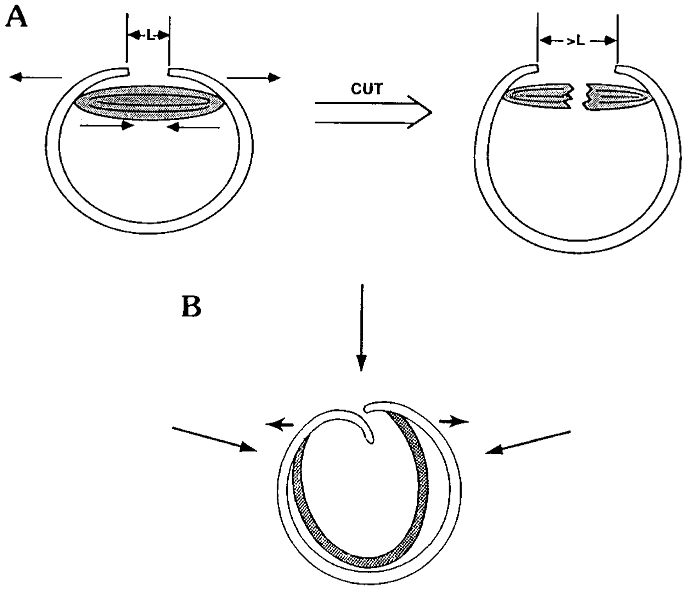

Fig. 5. Tracheal ring forces at rest and during compression. *A*, at rest, the cartilagenous ring exerts an elongating/outward force on the pars membranacea. *B*, during compression, extramural forces invaginate the pars membranacea and reduce the radius of curvature of the cartilagenous ring. An increase in the outward force of the ring occurs serving to limit the amount of compression.

the preterm (2, 3) and newborn (8) lamb and adult dog (15), and alterations in tracheal smooth muscle mechanics have been shown to parallel changes in tracheal compliance with development (4).

The role of tracheal cartilage in determining tracheal compliance has been less clearly defined. Moreno *et al.* (5) have demonstrated an increase in compliance in rabbit tracheae whose cartilage had been softened by papain administration. Before our study there were no data on age-related differences in the mechanical properties of tracheal cartilage or the extent to which these differences contributed to age-related differences in tracheal compliance.

The primary contribution of tracheal cartilage in determining tracheal compliance and dimensions appears to involve its role in limiting PM evagination (when transmural pressure is positive) and invagination (when transmural pressure is negative). When transmural pressure is zero (at rest) neither the PM nor the cartilagenous ring are at their true unstressed length. The cartilagenous ring exerts an elongating or outward force on the PM; the PM in turn exerts an equivalent force directed inward to establish an equilibrium (Fig. 5*A*). Gunst and Lai-Fook (6) measured this outward force at resting equilibrium to be 4 g/cm in the adult cat. During lung inflation, a decrease in intrathoracic pressure increases transmural pressure in the intrathoracic trachea, causing the PM to evaginate and the cartilagenous tips to be pulled further apart. The increasingly inward forces generated by the cartilagenous tips resist this expanding force, thereby limiting the increase in intraluminal area in the intrathoracic trachea. During exertional expiration, when intrathoracic pressures are relatively positive and transmural pressures in the intrathoracic trachea may be negative, PM invagination and tracheal compression is limited by an outward, and therefore stabilizing, force exerted by the ring (5). In the immature animal, a reduced ability of the cartilagenous ring to exert these forces may contribute to age-related differences in tracheal compressibility/inflatability.

Relative tensions of complex, cartilage in constant plane. Measurement of the length-tension characteristics of both the PM/cartilage complex and the cartilagenous ring enabled us to compare relative force generation from each component. In both the preterm (Fig. 3*A*) and adult (Fig. 3*B*) groups the slope of the length-tension line for the complex were greater than that of the cartilage alone. Because the PM and cartilage are in series, we conclude that the force generation in the PM exceeded that of the cartilage. This is not surprising because we know that at equilibrium (72%Lo in the preterm, 87%Lo in the adult) in the relaxed ring an outward force (a negative tension by our designation) is exerted.

Developmental comparison. To allow us to compare the mechanical properties of tracheae of animals at different ages, lengths were normalized to a percent of Lo and tensions to the passive tensions at Lo. With data normalized in this manner, slopes of lines describing the relationship between %TPTLo and %CTLo as a function of %Lo provided an index of stiffness suitable for statistical comparison.

Comparison of the slopes of both the complex and cartilage for preterm *versus* adult reveals significantly higher ($p < 0.05$) slopes in the adult for both cases, suggesting greater stiffness in the adult ring. Data points were linear throughout the range of lengths 70–117%Lo. Tension generation outside this range of length was extremely great and points deviated from linearity, particularly in the adult group. This result is consistent with the observations that biologic materials tend not to follow Hooke's law. In addition, alterations in the tension development in the cartilage reflect both an altered geometry as well as the inherent mechanical properties of the tissue.

Of further interest is the observation that in the resting state PM length in the preterm was only 72%Lo compared to 87%Lo in the adult. This difference may represent a developmental difference in the interplay between the outward force of the cartilage and the passive mechanical properties of the PM.

Physiologic significance. The physiologic significance of tracheal cartilage appears intimately related to its structural and mechanical properties. As a determinant of tracheal compliance, the stiffness of tracheal cartilage defines the limit of acute and chronic volume deformation that occurs with ventilation. In the extrathoracic trachea, cartilage may serve to maintain tracheal patency in the face of external compression by surrounding tissue. In addition, cartilage may help the trachea maintain longitudinal stability by preventing buckling during periods of extreme neck flexion or extension.

In the intrathoracic trachea, alterations in thoracic pressure that occur throughout the respiratory cycle will in part determine tracheal dimensions (and resistance to airflow) for a given tracheal compliance. During the course of passive expiration, when transmural pressure gradients in the trachea increase as lung volume approaches functional reserve capacity, the cartilagenous ring exerts an increasing outward force that helps to limit PM invagination and changes in tracheal cross-sectional area. McCormack *et al.* (7) have suggested that when this force is reduced by softening airway cartilage, the consequent reduction in cross-sectional area can produce an increase in expiratory resistance, even during tidal breathing.

During forced expiration the compliance and cross-sectional area of the flow-limiting segment of airway are the principal determinants of $\dot{V}$max. Both adult dogs (16) and rabbits (7) treated with proteolytic enzymes to soften tracheal cartilage have demonstrated significant reductions in $\dot{V}$max. Inasmuch as neither lung elastic recoil nor upstream resistance was altered in these models, the reductions in $\dot{V}$max can be attributed solely to alterations in central airway mechanics (7).

Similarly, high compliance of cartilage in immature tracheae may contribute to the severe flow limitation observed in premature infants. In adults, the stiffness of tracheal cartilage most likely precludes the existence of proximal, flow-limiting sites and serves to shift the appearance of these sites to more distal, compliant airways.

In summary, this study analyzed functionally relevant length-tension characteristics of premature and adult tracheal rings. Results demonstrate that both the ring complex as well as the cartilage alone are more compliant in the preterm compared to the adult sheep. These age-related differences parallel developmental differences observed in tracheal smooth muscle and tracheal mechanics. Age-related differences in tracheal compliance may therefore reflect an increase in stiffness that occurs in both components with maturation.

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