Aging increases upper airway collapsibility in Fischer 344 rats

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Ray AD, Ogasa T, Magalang UJ, Krasney JA, Farkas GA. Aging increases upper airway collapsibility in Fischer 344 rats. J Appl Physiol 105: 1471–1476, 2008. First published August 28, 2008; doi:10.1152/japplphysiol.00166.2008.—The upper airway muscles play an important role in maintaining upper airway collapsibility, and the incidence of sleep-disordered breathing increases with age. We hypothesize that the increase in airway collapsibility with increasing age can be linked to changes in upper airway muscle mechanics and structure. Eight young (Y: 6 mo) and eight old (O: 30 mo) Fischer 344 rats were anesthetized and mechanically ventilated, and the pharyngeal pressure associated with flow limitation (Pcrit) was measured 1) with the hypoglossal (cnXII) nerve intact, 2) following bilateral cnXII denervation, and 3) during cnXII stimulation. With the cnXII intact, the upper airways of older rats were more collapsible compared with their younger counterparts [Pcrit = −7.1 ± 0.6 (SE) vs. −9.5 ± 0.7 cmH2O, respectively; P = 0.033]. CnXII denervation resulted in an increase in Pcrit such that Pcrit became similar in both groups (O: −4.2 ± 0.5 cmH2O; Y: −5.4 ± 0.5 cmH2O). In all rats, cnXII stimulation decreased Pcrit (less collapsible) in both groups (O: −11.3 ± 1.0 cmH2O; Y: −10.2 ± 1.0 cmH2O). The myosin heavy chain composition of the genioglossus muscle demonstrated a decrease in the percentage of the IIb isoform (38.3 ± 2.5 vs. 21.7 ± 1.7%; P < 0.001); in contrast, the sternohyoid muscle demonstrated an increase in the percentage of the IIb isoform (72.2 ± 2.5 vs. 58.4 ± 2.3%; P = 0.001) with age. We conclude that the upper airway becomes more collapsible with age and that the increase in upper airway collapsibility with age is likely related to altered neural control rather than to primary alterations in upper airway muscle structure and function.

obstructive sleep apnea; pharyngeal pressure; upper airway muscle

OBSTRUCTIVE SLEEP APNEA (OSA), a highly prevalent condition that affects ~5% of the Western population, is associated with an increased risk of cardiovascular disease (18, 36). The condition is characterized by repetitive upper airway obstruction during sleep. The mechanisms leading to upper airway occlusion are incompletely understood and almost certainly multifactorial (32). Epidemiological studies have identified increasing age as an independent risk factor for the development of OSA (1, 4, 36). More importantly, older age has been associated with increased upper airway collapsibility during sleep in healthy human subjects, independent of gender and body mass index (9).

Aging is associated with changes to the structures surrounding the upper airway, which may contribute to the pathogenesis of OSA. Changes to the soft tissue surrounding the upper airway may include alterations in muscle structure and function, increases in parapharyngeal fat pad deposition, changes in sensation, and a reduced negative pressure reflex (17, 35). However, it is yet to be determined whether muscular and/or soft tissue adaptations secondary to the aging process predispose the upper airway to collapse.

Skeletal muscle has been shown to undergo structural and biochemical changes as a result of the aging process (28). Based on animal studies investigating the age-related changes to the upper airway muscles (UAM; genioglossus, sternohyoid, and geniohyoid), there is a general increase in the number of fast fatigable fibers (23, 29) and a decrease in upper airway muscle endurance (29).

Even though structural and metabolic changes have been demonstrated in the UAM secondary to aging, the in vitro contractile kinetics were reported to be altered in some (24, 29) but not all studies (7). Human studies have also demonstrated a decline in genioglossal activation and protrusion strength with age. Therefore, it is conceivable that a decrease in genioglossus muscle strength may alter airway stability and contribute to the increased incidence of OSA with aging (19, 33, 34).

The purpose of the present study was to investigate whether upper airway mechanics are altered with age. Utilizing an in situ upper airway preparation (20, 22, 25), we assessed maximal airflow and the upper airway closing pressure (Pcrit) in young and old Fischer 344 rats. Fischer 344 rats were selected, because, unlike other rat strains that gain weight with age, Fischer 344 rats show minimal to no weight gain with age, making them ideal for studies examining the effects of age on upper airway collapsibility. We hypothesize that aging is independently associated with a more collapsible upper airway and that age-related changes to the UAM contribute to the increase in airway collapsibility.

METHODS

The study was performed in eight young (6 mo) and eight older (30 mo) male Fischer 344 rats (National Institute on Aging, Bethesda, MD). Ambient temperature was maintained at 21°C, and an artificial 12-h light-dark cycle was set. Rats were provided standard laboratory chow (Ralston Purina, St. Louis, MO) and water ad libitum. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the University at Buffalo.

Upper Airway Mechanics

An isolated upper airway preparation previously utilized in our laboratory, described in more detail by Nakano et al. (20), was applied to determine the pharyngeal critical pressure (Pcrit), maximal inspira-

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tory airflow ($V_{IMAX}$), and oro-nasal resistance ($R_{on}$) (20, 22, 25). Animals were initially anesthetized with 4.0% isoflurane and placed supine on a circulating water heating pad. Rats were then mechanically ventilated (model 683; Harvard Apparatus, South Natick, MA) and continually anesthetized with 2–3% isoflurane. The trachea was transected caudal to the larynx, and end-tidal $CO_2$ was monitored continuously (Capstar-100; CWE, Ardmore, PA) through the caudal tracheal stub and maintained at 5% by adjusting the rate of the mechanical ventilator. A rigid cannula was placed in the rostral cut end of the trachea and tied into place. A mobile catheter with a side hole midway down its length was inserted through the caudal end of the rigid cannula and exited out one nostril. This catheter was used to measure the pharyngeal pressure ($P_{ph}$) at different locations in the upper airway. A second catheter was inserted into the rigid cannula to record the hypopharyngeal ($P_{php}$) pressure and was positioned lower in the airway. We could determine the flow-limiting site (FLS) by monitoring the shapes of the $V_{IMAX}$ vs. time and $P_{ph}$ vs. time curves. In brief, pressure was rapidly lowered in the downstream end of the upper airway. As $P_{ph}$ was lowered, inspiratory airflow rose and reached a maximum at the onset of the inspiratory airflow limitation followed by a decrease. Once the FLS was localized, the catheter was fixed at this position to measure $P_{ph}$. $P_{crit}$ was defined as the nadir in $P_{ph}$ at the onset of flow limitation as shown in Fig. 1. Resistance upstream to the flow-limiting site was defined as $R_{on}$. $R_{on}$ was calculated as follows: $R_{on} = (P_{on} - P_{crit})/V_{IMAX}$, where oronasal pressure ($P_{on}$) remained atmospheric. $P_{ph}$ and $P_{php}$ were monitored by pressure transducers (P23XL; Stratham Laboratories, Oxnard, CA). The inspiratory airflow through the airway was measured with a pneumotachograph (no. 0.771; Fleisch) and a differential pressure transducer (model MP 45-1, range 2 cmH2O, Validyne Engineering, Northridge, CA). Rectal temperature was monitored and maintained at 37°C with the aid of a heating pad. Atropine sulfate (0.4 mg/kg im) was administered to minimize airway secretions.

To determine the relative role of neural inputs in the maintenance of upper airway stability, we measured upper airway mechanics at baseline ($cnXII$ intact), following bilateral $cnXII$ denervation, and during bilateral $cnXII$ electrical supramaximal stimulation. $cnXII$ was dissected free bilaterally from where the nerves cross the carotid artery and were sectioned just proximal to the bifurcation into the lateral and medial branches. Mineral oil was applied to prevent nerve desiccation. Stimulating electrodes were connected to a constant current electrical stimulus isolation unit (model SIU8T, Grass Instruments, Quincy, MA) and stimulator (S44 Stimulator, Grass Instruments). An average of three measurements of $P_{crit}$, $V_{IMAX}$, and $R_{on}$ were obtained under three separate conditions: 1) baseline (uncut), 2) after $cnXII$ denervation, and 3) during $cnXII$ bilateral supramaximal stimulation (pulse duration of 0.1 μs, stimulation frequency of 100 Hz, and stimulation voltage of 40 V). At the completion of the experiment, the genioglossus, geniohyoid, and sternohyoid muscles were removed, quickly frozen in liquid nitrogen, and stored at −70°C for later biochemical analysis.

**Myosin Heavy Chain Analysis**

Myosin heavy chain (MHC) isoforms from the genioglossus, geniohyoid and sternohyoid muscles were separated using SDS-PAGE using a mini-gel system (Bio-Rad, Hercules, CA). The gels consisted of a 4% acrylamide (30% glycerol, pH 6.8) stacking gel and an 8% acrylamide resolving gel (30% glycerol, pH 8.8). The samples were run at a constant voltage (150 V) for 20–24 h at 5°C. After electrophoresis, the gels were silver stained according to a technique by Oakley (21). A digital image was then taken of the gel, and the relative distribution of each isoform (I, 2a, 2b, and 2x) was determined by densitometry as previously described (11).

**Statistical Analysis**

The differences in upper airway mechanics were analyzed with a two-way ANOVA with repeated measures. The between-subject factors were young vs. old for all measurements, and the within-subject factors were base vs. cut vs. stimulation (upper airway mechanics). If

![Fig. 1. A representative tracing from a young Fisher-344 rat depicting inspiratory airflow ($V_i$), pharyngeal pressure ($P_{ph}$), and hypopharyngeal pressure ($P_{php}$) in an isolated upper airway preparation. $P_{php}$ was measured at or immediately upstream to the flow-limiting site. As $P_{ph}$ was lowered, $V_i$ rose and reached a maximum ($V_{IMAX}$) at the onset of inspiratory airflow limitation. $P_{crit}$ was defined as the nadir in $P_{ph}$ vs. time curve. $P_{crit}$ increased (more collapsible) after bilateral $cnXII$ transection. Bilateral electrical stimulation of $cnXII$ decreased $P_{crit}$ (less collapsible).](./image.png)
statistical significance existed, a post hoc analysis with Bonferroni corrections was used to determine the basis for the differences. Age-related changes to each individual MHC isoform (I, IIA, IX, and IIB) were also analyzed using a two-way ANOVA with repeated measures. If statistical significance existed, Bonferroni corrections were used to determine the differences. A P value of <0.05 was considered significant. All data are presented as means ± SE.

RESULTS

Body Weight

The younger, 6-mo-old Fischer rats were significantly heavier (420 ± 4 g; range: 406–432 g) than the older, 30-mo-old Fischer rats (384 ± 12 g; range: 347–452 g; P = 0.01).

Upper Airway Mechanics

Baseline. Upper airway mechanics are displayed in Fig. 2. During baseline recordings, Pcrit was significantly greater (less negative or more collapsible) in the old rats compared with the young rats (P < 0.05), and despite a more stable upper airway, Ron was elevated in the young compared with the old rats (P < 0.05). However, there were no differences in V˙IMAX between the two age groups.

CnXII denervation. In the young rats, bilateral cnXII denervation increased (Pcrit more positive) airway collapsibility and decreased V˙IMAX and Ron from baseline conditions (P < 0.05). Similarly, bilateral cnXII denervation significantly increased Pcrit and decreased V˙IMAX and Ron from baseline conditions in old rats (P < 0.05). Pcrit, Ron, and V˙IMAX were not different between the two age groups following cnXII denervation.

Hypoglossal nerve stimulation. Supramaximal stimulation of cnXII significantly decreased Pcrit, increased V˙IMAX, and increased Ron compared with cnXII denervation trials in the young rats (P < 0.05). However, compared with baseline conditions, there were no differences in Pcrit, V˙IMAX, and Ron. Therefore, supramaximal stimulation of cnXII restored baseline conditions in the young rats.

In the old rats, Pcrit was significantly decreased, whereas V˙IMAX and Ron were increased compared with cnXII denervation (P < 0.05). In contrast to the young rats, old rats also demonstrated significant differences between cnXII stimulation and baseline conditions with respect to Pcrit, V˙IMAX, and Ron (P < 0.05). Despite the differences between baseline and cnXII stimulation in the old rats, there were no differences in Pcrit, V˙IMAX, and Ron during cnXII stimulation compared with the young rats (Fig. 2).

MHC

Figure 3 represents the individual MHC isoforms isolated from the genioglossus, geniohyoid, and sternohyoid muscles from young and old rats. Combined and not dependent on age, all three UAM are composed primarily of fast, type II isoforms, expressing minimal or no type I isoforms. With age, the genioglossus muscle decreased the percentage of the IIB isoform (P < 0.001), while increasing the percentage of the IIA (P < 0.001) and the IX isoform (P < 0.005). The sternohyoid muscle demonstrated an increase in the percentage of the IIB isoform (P = 0.001) and a decrease in the percentage of the IX isoform (P < 0.001). In contrast to the other two muscles, the relative percentage of the MHC isoforms isolated from the geniohyoid muscle did not change with age.

DISCUSSION

The major findings from the current study are that, compared with young rats, 1) Pcrit is elevated (more positive) indicative of a more collapsible upper airway in old rats; 2) structurally, absent of neuromuscular influences (cnXII denervation), the intrinsic collapsibility of the upper airway is stable with age; 3) bilateral stimulation of the hypoglossal nerves improved airway collapsibility to levels that surpass baseline conditions...
only in the old rats; and 4) aging resulted in adaptations to the UAM that did not reflect changes to airway collapsibility during supramaximal stimulation.

Upper Airway Collapsibility

Despite age-related changes to the UAM, cnXII stimulation was capable of improving upper airway collapsibility above baseline values in old Fischer rats. This response has also been reported in other strains of rats (10, 20, 25) and cats (27). In addition, cnXII stimulation resulted in small increases in $V_{\text{IMAX}}$ and $R_{\text{on}}$; again a response that has been observed in previous studies (10, 27). We suggest the increase in $V_{\text{IMAX}}$ was the result of a more stable upper airway, whereas the improvements in airflow were partially offset by a simultaneous increase in $R_{\text{on}}$ cephalad to the flow-limiting site.

Because $V_{\text{IMAX}}$ is determined by $P_{\text{crit}}$ and $R_{\text{on}}$ during flow limitation, it is suggested that the increase in $R_{\text{on}}$ was due to a narrowing of the cephalic segment of the upper airway (27). However, the ability to improve airway collapsibility during stimulation indicates that the UAM have the ability to produce sufficient force despite age-related adaptations.

Collapsibility of the upper airway is controlled through reflex mechanisms. Mucosal receptors and afferents within the upper airway respond to the negative pressures created during inspiration, reflexively activating the UAM. It is possible that impairment of the upper airway pressure receptors may lead to reductions in airflow (hypopnea) or complete airway collapse (apnea). Previous studies have shown a decrease in laryngopharyngeal sensitivity to air pulse stimulation in the elderly (2, 3) and OSA patients demonstrate a deficiency in vibratory and two-point discrimination in the upper airway (14); these age- and disease-related decreases in sensation may be due to morphological changes in the number of sensory structures, including laminar nerve endings, which are believed to be sensitive to changes in upper airway pressure (35). Because the older Fischer rats (baseline conditions) did not respond to negative pressures to the same extent as their younger counterparts, it is difficult to distinguish between altered UAM function or to attenuation of the negative pressure reflex arc or due to reduced receptor sensitivity or altered central processing. However, there were no differences in $P_{\text{crit}}$ between age groups during bilateral cnXII denervation. Moreover, the denervated experiments provide additional support that the inherent stability of the airway musculature does not differ with age. The combined neural denervation and controlled stimulation experiments provide support for the possibility that an age-related decline in the activation of the negative pressure reflex may have contributed to the increase in airway collapse in the old Fischer rats. Additional studies, however, are needed to address this point.

Aging and Upper Airway Muscle

Skeletal muscle undergoes structural and biochemical changes as a result of aging (28), which may have contributed to the increase in upper airway collapsibility in the older Fischer rats. With advancing age, limb muscle undergoes type II fiber atrophy, decreases in cross-sectional area, force production, and shortening velocity (6, 16). In addition, MHC (types I, IIa, IIx, and IIb) isolated from limb muscle also demonstrate a transition from faster (type II, increased force production and more fatigable) to slower (type I, decrease force production and less fatigable) isoforms with advancing age (6). The MHC composition of muscle is analyzed because it correlates to the velocity of shortening and to the peak isometric force produced by the muscle (8). In addition, the relative proportion of each MHC isoform is correlated to the area occupied by the corresponding muscle fiber (15).

In contrast to limb muscle, ventilatory muscles demonstrate atrophy in the slower, more oxidative type I fibers with age, whereas Gosselin et al. (12) demonstrated a slow to fast MHC isoform transformation in the diaphragm muscle isolated from 24-mo-old Fischer rats. Thus age-related muscle adaptations seem to be dependent on the action of each muscle and in the event such changes may occur, it remains to be determined what functional impact aging would have on the UAM.

The current study demonstrates contrasting results with respect to aging muscle. The genioglossus muscle demonstrated a transition from the faster IIb MHC isoform to the more oxidative IIa and IIx isoforms in the old Fischer rats. These muscular adaptations are in contrast to Oliven’s work in the genioglossus muscle but consistent with a decline in the...
type IIb MHC isoforms noted in aging limb muscle (23). A shift in this direction may result in a decrease in force production and an increase in recovery time, rendering the upper airway more susceptible to collapse.

In contrast, the sternohyoid muscle demonstrated an increase in the percentage of the type IIb MHC isoform, a change that is consistent with previous studies in the aging diaphragm (12). Similar changes to the sternohyoid and geniohyoid muscles were also shown in a study by van Lunteran et al. in 20- to 21-mo-old Fischer rats (29). In that study, van Lunteran also demonstrated an age-related increase in the cross-sectional area and the number of IIb fibers. The overall goals of the UAM are to decrease airflow resistance, and the mechanisms by which they improve airway stability are different based on their anatomical location, i.e., the SH muscle moves the hyoid bone caudally to widen the larynx (13). In addition, the UAM are active during nonventilatory maneuvers such as postural movements, exploratory behavior, grooming, thermoregulation, and nutrition (31). It is possible that a change in these behaviors may also influence UAM morphology with age. Thus it is possible that an increase in the number of fast fatigable fibers in one muscle could impact other muscles in close proximity and/or with a similar function.

Critique of Methodology of Isolated Upper Airway

The application of the isolated in situ upper airway preparation allowed us to measure the UA closing pressure (Pcrit) in a rodent model. However, the current study has several limitations that need to be addressed. First, the young Fischer rats were significantly heavier than those from the older group. Current literature suggests that obesity influences upper airway dynamics, and weight loss has been shown to improve airway stability in patients with OSA (26). Brennck et al. (5) demonstrated that the obese Zucker rat, an animal model of early onset genetic obesity, exhibits a decreased diameter and cross-sectional area of the upper airway. Consistent with these findings, we reported that obese Zucker rats have a more collapsible upper airway compared with their age-matched lean counterparts (20, 25). However, in contrast to our previous studies in obese Zucker rats, the present study demonstrated that younger, significantly heavier Fischer rats have a more stable UA compared with their older, lighter counterparts. Albeit significant, the differences in body weight were not a contributing factor to the present study, and therefore our conclusions regarding the increased upper airway collapsibility with age are valid. Second, we did not seal the mouth closed during the procedure, and there is a possibility that airflow dynamics could be altered because of an open mouth. However, utilizing a similar approach, Fuller et al. demonstrated that Perit and $V_{\text{IMAX}}$ were not dependent on whether the mouth was open or closed (10). In an attempt to standardize the animal’s position, we maintained a neutral head and neck angle throughout the testing procedures. Third, large negative suction pressures ($\sim$ 150 cmH$_2$O) were generated to achieve airflow limitation. These large negative pressures were outside the physiological range and could conceivably traumatize the UA, leading to alterations in airway stability. However, repeated measures of UA collapsibility were reproducible with little variation. Last, it is possible that establishing an end-tidal CO$_2$ equal to 5% may not have completely eliminated UAM activity during baseline measurements; as a result, there may have been differences in baseline muscle activity between groups of animals.

In summary, the present study demonstrates that age-related adaptations to the UAM do not alter the in situ collapsibility of the upper airway during bilateral cnXII stimulation in 30-mo-old Fischer rats. Therefore, we suggest factors other than intrinsic structural and functional changes are responsible for the increase in airflow collapsibility in the old Fischer rats. Other potential contributing factors include altered neurologic control of the UAM. Veasey et al. (30) demonstrated that altered neurologic control of the UAM, rather than muscular adaptations, may be the primary mechanism controlling airway stability in an animal model of OSA. Therefore, we suggest age-associated factors that alter central neural control of the upper airway may be important contributors to upper airway collapsibility in the elderly and suggest that additional studies are warranted to address the importance of the aging upper airway and the potential confounding factors associated with the neurologic control of the aging upper airway.

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