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Healthy humans with a narrow upper airway maintain patency during quiet breathing by dilating the airway during inspiration

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Key points

- During quiet breathing, the muscles of the upper airway of healthy humans contract to dilate the airway during inspiration.
- We used dynamic tagged magnetic resonance imaging to show that the amount of active upper airway dilatation during inspiration increases as airway cross-sectional area decreases.
- Older and more overweight subjects have smaller airways, and this is linked to increased active airway dilatation.
- These data show that healthy subjects with narrow airways can overcome anatomical risk factors for airway collapse by actively dilating their airways during inspiration.
- In contrast, obstructive sleep apnoea patients with similarly narrow airways show either little or no dilatory motion during inspiration or abnormal bi-directional movement. This regulation of airway behaviour fails in these patients.

Abstract A patent upper airway is essential for survival. Increased age, obesity and some upper airway anatomical features are associated with failure to maintain upper airway patency during sleep, leading to obstructive sleep apnoea. However, many healthy subjects with these risk factors do not develop this condition. The aim of this study was to determine how anatomical factors and active dilator muscle contraction contribute to upper airway patency in healthy volunteers across a broad range of age and body mass index (BMI). A 'tagged' magnetic resonance imaging technique quantified respiratory-related motion of the anterior and lateral walls of the upper airway during quiet breathing in the supine position. Fifty-two subjects aged 22−68 years with BMI from 17.5 to 40.1 kg m^{-2} were studied. Higher BMI was associated with smaller airway cross-sectional area at the level of soft palate ($P < 0.05$). The genioglossus moved anteriorly to dilate the upper airway during inspiration. This movement increased with increasing BMI, increasing age, a smaller airway area, and steeper tongue-base angle (all $P < 0.05$). Motion of the lateral upper airway at the soft-palate level was variable and less strongly linked to anatomical features of the upper airway. Multiple regression indicated that anterior genioglossus motion decreased with increasing airway area ($P = 0.03$) and with increasing tongue-base angle ($P = 0.02$). These data suggest that healthy humans, including those whose anatomy places them at increased risk of airway closure, can maintain upper airway patency by dynamically dilating the airway during inspiration.

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Abbreviations BMI, body mass index; MR, magnetic resonance; MRI, magnetic resonance imaging; OSA, obstructive sleep apnoea.

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Introduction

Maintenance of upper airway patency during wakefulness and sleep is a dynamic process which is essential for survival. Patency of the upper airway relies on a balance between forces that tend to dilate the airway (e.g. dilator muscle activity) and those that act to collapse it (e.g. negative airway pressure during inspiration). At the airway wall, the local balance between the tissue stresses that act radially (towards the airway) and airway pressure governs whether the airway dilates or narrows. In the supine position, gravity acts to push the genioglossus posteriorly, narrowing the airway, but this force is constant, while other forces such as active muscle contraction (e.g. Mezzanotte *et al.* 1996) and airway pressure vary during the respiratory cycle (Schwartz *et al.* 1988).

In obstructive sleep apnoea (OSA), the upper airway collapses repeatedly during sleep, and arousals and increased contraction of airway dilator muscles are required to reopen the airway and establish airflow. These repeated arousals are associated with daytime sleepiness and cardiovascular sequelae (Yaggi *et al.* 2005), although the mechanism of the latter are not yet well understood. Several factors predispose individuals to airway collapse during sleep, including obesity (Rubinstein *et al.* 1988; Foster *et al.* 2009; Schwartz *et al.* 2010) and craniofacial characteristics that result in a narrow airway (Schwab *et al.* 1995), along with altered neural control of the upper airway (Malhotra *et al.* 2002). While it is clear that OSA can be generated by different peripheral and central mechanisms (e.g. Eckert & Younes, 2014), many healthy individuals are able to maintain airway patency during sleep despite having one or more major risk factors for OSA. However, the mechanisms by which this is achieved in different individuals are not known. Few studies have examined how the healthy upper airway responds mechanically during breathing, and how this varies with age, obesity and other risk factors for OSA. While other researchers have examined changes in 2-D airway calibre in OSA subjects in response to applied pressures awake (Kuna *et al.* 1988) or asleep (Launois *et al.* 1993), or compared OSA and control subjects (Schwab *et al.* 1993*a*) using a range of imaging techniques, only one study has examined how airway size changes during quiet breathing in healthy subjects (Schwab *et al.* 1993*b*). Using dynamic computed tomography (CT) imaging, their study revealed that there was little change in airway area during inspiration in quiet breathing in healthy weight subjects, but did not examine the effect of airway size, age or obesity on airway dynamics. Such information would not only provide insight into the normal physiology of the human upper airway but it may shed light on the pathophysiology of OSA.

We previously used 'tagged' magnetic resonance imaging (MRI) to quantify the usual respiratory motion of the genioglossus and surrounding upper airway during quiet breathing (Cheng *et al.* 2008). In a small group of young healthy adults ($n = 6$) the posterior region of genioglossus moved anteriorly during inspiration to dilate the upper airway with little motion in adjacent regions. Subsequently, we showed that this motion began prior to airflow and it was affected in a predictable way by increasing the negative pressure in the airway: inspiratory anterior motion diminished with greater negative pressure (Cheng *et al.* 2011). Recent studies have revealed different patterns of upper airway motion during quiet breathing between healthy subjects and OSA patients who were matched for age and body mass index (BMI) (Brown *et al.* 2013). However, it is not known how healthy subjects continually maintain airway patency despite risk factors for OSA, such as obesity. Therefore, this study aimed to determine the influence of age, obesity and quasi-static anatomical features of the upper airway on dynamic motion of the upper airway in healthy human subjects. The anatomical factors chosen for study are the key parameters that define air flow rate in the pharynx, including airway minimum cross-sectional area, and pharyngeal length. We hypothesised that airway patency in healthy subjects with risk factors for OSA is maintained by active dilatation of the upper airway muscles during inspiration.

Methods

Subjects

Fifty-two healthy subjects (31 males) with no history of major cardiorespiratory or sleep disorders were recruited. All subjects were screened for excessive daytime sleepiness using the Epworth Sleepiness Score (ESS), and contraindications to MRI. Inclusion criteria were either an ESS less than 10 (for subjects who did not undergo a polysomnogram) or an apnoea–hypopnoea index (AHI) less than ten, for a subset of subjects ($n = 18$) who had undergone a clinical polysomnogram as part of their clinical care. The subjects were aged 22–68 years and had body mass indices (BMI) ranging from 17.5 to 40.1 kg m^{-2}. Subjects with both normal and high BMIs across the age span were targeted for recruitment. Some subjects were participants in previous studies of upper airway motion using identical imaging protocols ($n = 22$, Cheng *et al.* 2008; Brown *et al.* 2013). However, all imaging data were completely reanalysed for this study. The study was approved by the Human Research Ethics Committee of the University of New South Wales, and conducted according to the *Declaration of Helsinki*. Informed written consent was obtained from all subjects.

Imaging protocol

Data were acquired using a 3 tesla MRI scanner (Philips Achieva TX, Best, The Netherlands) at the Diagnostic

MRI Services facility at Neuroscience Research Australia using an eight-channel transmit–receive neurovascular coil. Subjects lay supine on the scanner bed, and were asked to breathe quietly through their nose. Head position was standardised to reduce variation in upper airway size due to posture. The Frankfort plane (a plane from the lateral edge of the orbit to the superior portion of the tragus) was perpendicular to the scanner table. Foam pads around the head minimised head motion. An MRI-compatible respiratory sensor (Philips Medical, Best, The Netherlands) was used to monitor respiration. We communicated with subjects throughout the scan to ensure that they were awake, and this was confirmed after the scan by verbal report.

A 2-D complementary spatial modulation of magnetisation (CSPAMM) imaging sequence was used to image genioglossus motion in the mid-sagittal plane, and lateral airway wall motion in the axial plane at the level of the soft palate (Fig. 1). This method has been described previously (Cheng *et al.* 2008, 2011) and is summarised here. Briefly, around 140 ms before image acquisition, a rectilinear grid was 'tagged' on the tissue by saturating the applied radiofrequency in a spatially selective manner. Images of the tagged tissue were then taken at 250 ms intervals until the grid had faded from the region of interest (~1000 ms after the initial tag). The tagged grid was then reapplied repeatedly to image the respiratory cycle. As this sequence requires a cardiac signal, an artificial ECG trigger was generated using LabVIEW v8.2 (National Instruments Corp., Austin, TX, USA). The scanner was triggered prior to inspiration to cover the inspiratory phase of the respiratory cycle. The imaging parameters used were: flip angle 90 deg; repetition time 400 ms; echo time 16 ms; 256 × 256 matrix with a slice thickness of 10 mm (pixel size 0.86 mm × 0.86 mm for sagittal scans and 0.78 mm × 0.78 mm for axial scans), and tag spacing 8.6 mm. Tissue motion was imaged in both planes over an average of three respiratory cycles for each subject. In addition to the tagged images, T1-weighted anatomical images of the upper airway were also acquired using 3-D turbo spin echo technique (matrix 256 × 256, 130 slices, 1 mm isotropic, TR = 5.9 ms, TE = 2.64 ms).

Image analysis

The tagged image sequences were reviewed and sequences including swallowing or mandibular movement of more than 1 mm were discarded. Genioglossus motion was calculated during post-processing using the harmonic phase method (HARP) (Osman *et al.* 1999). HARP uses the principle that tissue deformation alters tag spacing in the image, and this is quantifiable from changes in the spatial frequency of the tagged grid in the Fourier domain. This technique gives sub-pixel resolution with a displacement error of 0.1 pixels (Kerwin & Prince, 2000). Average anterior genioglossus and lateral wall motion during inspiration (beginning just prior to inspiration) were calculated over three respiratory cycles. Genioglossus motion was analysed at a point 10 mm anterior to the epiglottis. Lateral wall motion was analysed at two points, 10 mm to the left and right of the airway wall (Points B and C, respectively of Fig. 1*B*). In addition, anatomical measurements were made (shown in Fig. 1), including the cross-sectional area of the pharynx at the level of the soft palate. Pharyngeal length is defined by the vertical distance between the hard palate and the tip of the epiglottis, in the vertical image direction. The tongue-base angle is defined by the angle between the inferior margin of the geniohyoid and the posterior pharyngeal wall.

Statistical analysis

A linear regression analysis was performed on the interrelationships between BMI, age, cross-sectional pharyngeal area, tongue-base angle, pharyngeal length and soft tissue motion (upper airway motion). A 95% confidence interval for the coefficient (slope $\boldsymbol{\beta}$) of the univariate analysis was calculated. Units for the various slopes can be derived from Figs 3–7. Heteroscedasticity (i.e. whether the variance was consistent across the data) in the data was checked using the Goldfeld–Quandt test. Linear regression was used to assess the relationship between motion and age and BMI, and statistical significance was accepted at the 5% level. As the data were heteroscedastic (i.e. unequal variance across the range of the data), multiple linear regression was performed using bootstrapping methods (with 1000 samples) which do not assume homoscedasticity (equal variance). Moreover, as the relationship between pharyngeal cross-sectional area and genioglossus anterior motion was non-linear, a logarithmic transform was applied to the measure of genioglossus motion data prior to the multiple regression. All statistical analyses were performed using SPSS (IBM SPSS statistics, version 19).

Results

Sample tagged images depicting tongue anterior motion during inspiration are shown in Fig. 2, for typical low BMI and high BMI subjects. The first image was taken immediately before inspiration and the second 750 ms later.

Relationships between age, BMI and anatomical features of the upper airway

The recruitment strategy was designed to ensure a broad distribution of healthy subjects across the ranges of age

and body mass index (BMI). Figure 3 shows that this was achieved. Furthermore, there was no correlation between BMI and age in our group of 52 subjects ($P = 0.28$).

Figure 4 shows the relationship between BMI and pharyngeal area, tongue-base angle and pharyngeal length for the group of subjects. Pharyngeal cross-sectional area was measured at the soft palate level. Tongue-base angle reflected the sagittal angle between the geniohyoid and the pharyngeal wall. The pharyngeal area and tongue-base angle decrease with increasing BMI (slopes $\beta = -4.6$ and $\beta = -1.1$, respectively, $P < 0.00001$). Units for the slopes can be derived from the figures. There was no correlation between pharyngeal length and BMI ($\beta = -0.49$, $P > 0.05$).

Figure 5 shows the relationships between age and pharyngeal area, tongue-base angle and pharyngeal length. The pharyngeal area and tongue-base angle decrease as age increases (slopes $\beta = -1.09$ and $\beta = -0.21$, respectively; $P = 0.003$ and 0.036, respectively). The slope of the age and pharyngeal length relationship is also significantly different from zero ($\beta = 0.1$, $P = 0.038$). This indicates that pharyngeal length increases with age.

Relationship between age, BMI, upper airway anatomy and movement

BMI and upper airway inspiratory movement. Figure 6*A* shows the relationship between BMI and the anterior movement of the genioglossus during inspiration. The magnitude of this movement increased linearly with BMI ($\beta = 0.08$, $P < 0.0001$). Across the group, as BMI increased there was increased variability in the amount of inspiratory movement of the genioglossus. The Goldfeld–Quant test confirmed the heteroscedastic relationship ($P < 0.05$).

Figure 6*B* shows the relationship between BMI and the inspiratory movement of the lateral walls of the pharynx at the level of the soft palate. As for anterior movement of the genioglossus, the magnitude of outward lateral wall motion at the narrowest point of the nasopharynx increased with BMI ($\beta = 0.06$, $P = 0.009$).

Age and upper airway inspiratory movement. Figure 7 shows the relationship between age and movement of the upper airway tissues during inspiration. Anterior movement of the genioglossus during inspiration increased significantly with age ($\beta = 0.02$, $P = 0.006$). However, no relationship existed between age and the movement of the lateral airway walls ($\beta = 0.01$, $P = 0.22$).

Anatomical features and upper airway motion. Figures 8 and 9 show the independent linear regression analyses for pharyngeal area, tongue-base angle and pharyngeal length on upper airway motion. Figure 8*A* shows that anterior genioglossus movement decreased non-linearly with increasing pharyngeal area. That is, greater anterior movement occurred in subjects with smaller airways. To test the significance of the slope for pharyngeal area and genioglossus motion using linear regression, the square root of the airway area was used as the independent variable (notional airway radius). This revealed a significant, linear relationship between notional airway radius and genioglossus movement ($\beta = -0.02$, $P = 0.0002$; Fig. 8*B*). Genioglossus motion decreased with increasing tongue-base angle as shown in Fig. 8*C* ($\beta = -0.003$, $P = 0.001$). Of all the anatomical factors measured, pharyngeal length was the only factor which did not affect genioglossus movement ($P = 0.7$; Fig. 8*D*). Results for males and females were similar (Fig. 8*A*).

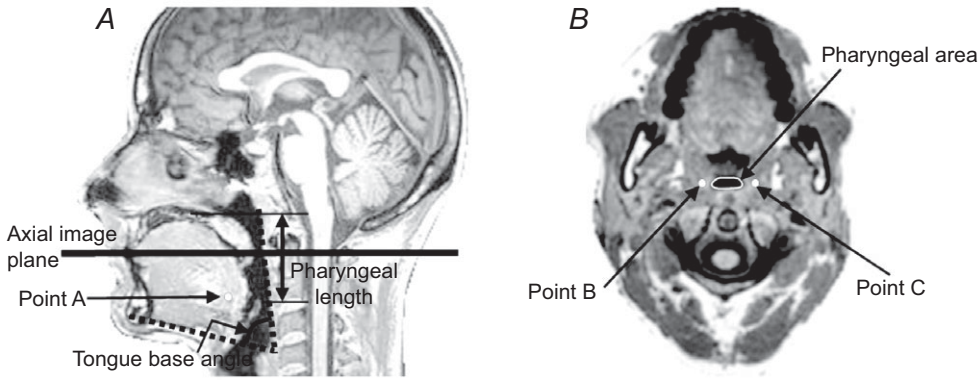

Figure 1. Location of sites of major anatomical measurements
A, sagittal MR image shows tongue-base angle (angle between inferior margin of geniohyoid and posterior pharyngeal wall); pharyngeal length (distance between hard palate and the tip of the epiglottis between thin black lines) and Point A used to quantify genioglossus motion. The thick black horizontal line indicates the location of the axial view used to measure cross-sectional area and movement of the lateral walls. *B*, axial image at the level of the soft palate, through the narrowest point of the nasopharynx showing the cross-sectional area of the pharynx. Points B and C were used to quantify motion of the lateral wall.

As shown in Fig. 9, tongue-base angle was the only anatomical factor significantly related to lateral wall motion, with decreasing motion associated with increasing tongue-base angle ($\beta = -0.04$, $P = 0.0005$). That is, the width of the airway increased more during inspiration in subjects with steeper tongue-base angles. The slopes of the relationships between lateral wall motion and the cross-sectional area of the pharynx, and between wall motion and pharyngeal length were not significantly different from zero ($P = 0.11$, $P = 0.93$, respectively).

Regression modelling

Multiple linear regression modelling of the measured physiological factors using bootstrapping (see Methods) showed that the cross-sectional area of the airway and BMI were highly collinear variables (Fig. 4*A*) and thus both could not be included in the regression model. As a result, BMI was omitted from the model. The remaining three variables (age, pharyngeal length and airway cross-sectional area), and the tongue-base angle were included in the initial model. After backwards selection to identify the most efficient model, only airway area and tongue-base angle remained in the model. Both were significantly related to the logarithm of anterior genioglossus motion. Anterior genioglossus motion decreased with increasing airway area (slope = −0.004, 95% confidence interval (CI) = −0.007 to −0.001), $P = 0.029$) and with increasing (i.e. more horizontal) tongue-base angle (slope = −0.013, 95% CI = −0.023 to −0.002, $P = 0.022$).

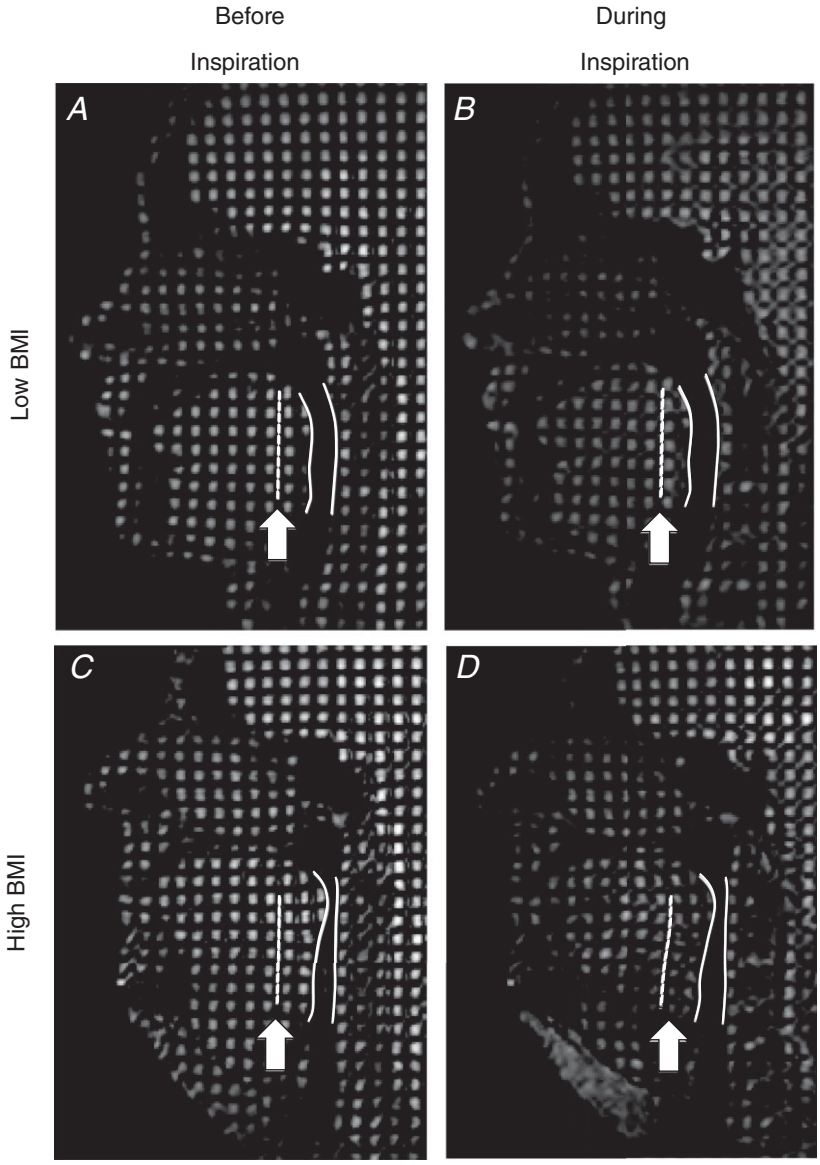

Figure 2. Examples of anterior tongue dilatory motion during early inspiration in a low BMI subject (upper panels) and high BMI subject (lower panels)
The left column (*A* and *C*) shows the undeformed tagged image just before inspiration, and the right column (*B* and *D*) shows the tagged grid 750 ms later during inspiration. In the low BMI subject (*A* and *B*) with a larger airway cross-sectional area (airway lumen is indicated by continuous white lines), there is little anterior motion (see white arrows) or deformation of the grid (see dashed lines). In the higher BMI subject (*C* and *D*) with a narrower airway, there is a clearly visible anterior motion of the the tongue (see white arrows) and deformation of the grid (see curved dashed vertical line).

Table 1. Summary of the interrelationships between physiological and anatomical factors and tissue motion during inspiration

	Anatomical variables			Upper airway motion	
	Pharyngeal area	Tongue-base angle	Pharyngeal length	Anterior genioglossus movement (inspiration)	Lateral wall motion (inspiration)
BMI	↓	↓	NS	↑	↑
Age	↓	↓	NS	↑	NS
Pharyngeal area	—	—	—	↓	NS
Tongue-base angle	—	—	—	↓	↓
Pharyngeal length	—	—	—	NS	NS

'NS' denotes that the regression slope was not significantly different from zero, '—' denotes not tested. Upwards arrow indicates that the column variable increases with increases in the row variable. Downwards arrow indicates that the column variable decreases with increases in the row variable.

Discussion

This study has identified a key new relationship between static anatomy of the upper airway and its active dilatation in healthy humans. Using dynamic tagged MRI, we found that the degree of active upper airway dilatation during inspiration increases as airway cross-sectional area decreases. That is, in healthy subjects with a narrow airway, the genioglossus contracts, moves anteriorly and dilates the upper airway during inspiration. However, in subjects with a larger upper airway, little anterior movement occurs. Higher BMI was associated with a narrower upper airway and thus larger airway dilatation of both the anterior and lateral walls of the upper airway during inspiration. There was also more anterior upper airway dilatation in subjects in whom the base of the genioglossus was more acutely angled to the airway wall than in those in whom the base of the tongue was more perpendicular to the airway wall. Motion of the lateral walls during inspiration was more variable, and was not strongly linked to anatomical features of the upper airway. Age was not strongly linked to airway wall motion during quiet breathing.

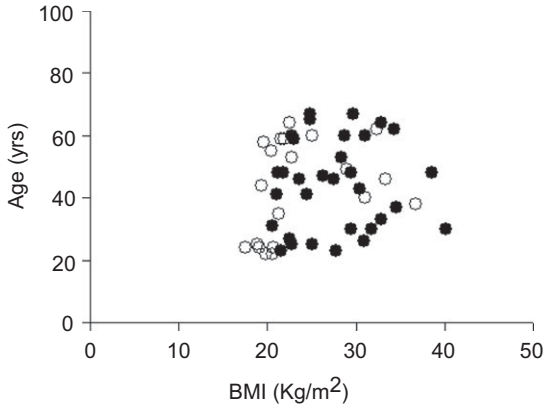

Figure 3. Relationship between BMI and age
Data are shown for females (open circles) and males (filled circles). There is no significant relationship between BMI and age (years) of the subjects ($P = 0.28$).

These results suggest that one mechanism by which healthy subjects overcome an intrinsically narrow airway is by active dilatation of the upper airway during inspiration. This dilatation would oppose the tendency of the negative inspiratory pressure in the pharynx to pull the airway closed. The current data suggest that there may be a threshold airway cross-sectional area, of approximately 60–70 mm^2, below which significant active anterior dilatation (> 1 mm) is required to maintain airway patency during quiet breathing in the supine position. The relatively small airway dilatation seen here in healthy weight control subjects is consistent with observations by Schwab *et al.* 1993*b* using dynamic CT. The mechanisms underpinning the larger dilatory movement in subjects with smaller airways are not yet clear. One potential mechanism is that subjects with narrower airways might have slightly more negative pressure in the airway during inspiration as a result of increased airway resistance (Owens *et al.* 2012), which in turn elicits enhanced reflex dilator muscle activity (e.g. Horner *et al.* 1991; Akahoshi *et al.* 2001). However, a previous study showed that when an external resistive load was imposed during inspiration, dilatory movement *decreased* in young lean subjects (Cheng *et al.* 2011). This was postulated to reflect a lower pressure in the airway which shifted the balance of forces between active dilatation and (negative) airway pressure. In the current study, however, the pressure in the airway is likely to be only slightly lower than in those with larger airways (Owens *et al.* 2012). It is unclear if this could be sufficient to evoke an increased dilatory response (see Fogel *et al.* 2003). However, it may be that the reflex evoked from focally narrow regions of the upper airway is particularly effective in producing dilatation. It has also recently been shown that obese healthy subjects have a

stronger muscle activation in response to a drop in airway pressure during sleep than either obese OSA patients and normal BMI healthy subjects (Sands *et al.*, AJCCRM 2014 (in press).

Some anatomical features were inter-related. Subjects with higher BMI tended to have both a narrower airway and a more angled tongue-base. This is consistent with previous anatomical studies (e.g. Mayer *et al.* 1996). It seems likely that fat deposition in the tongue which displaces the posterior tongue caudally contributes to the more angled tongue-base (see Isono, 2012 for review). This change in angulation of the infero-posterior muscle fascicles of genioglossus is also likely to reduce their 'mechanical advantage' for airway dilatation, as less of the shortening in a contraction moves the airway wall anteriorly, and more is directed vertically. Despite this, these subjects still had more anterior dilatation during inspiration than leaner individuals.

Within the group, the inspiratory movement of the lateral wall of the upper airway was more variable than in the anterior movement of the genioglossus. In some subjects, there was inward lateral wall movement (i.e. airway narrowing) during inspiration, in contrast to the dilatory motion of the genioglossus. Thus, the balance of forces at the airway wall differs for the lateral and anterior airway. This is consistent with their anatomically separate muscle structures and measurements of tissue pressure in rabbits (e.g. Kairaitis *et al.* 2009). Lateral wall movement increased with obesity, although the correlation with BMI was weaker than for anterior dilatation, and there was no association with pharyngeal area. A more angled tongue base was associated with more lateral wall dilatation, but not with other anatomical variables. The reasons for this are unclear.

Obesity increases neck circumference and fat deposition around the upper airway (e.g. Katz *et al.* 1990; Chi *et al.* 2011), and central obesity is linked to reduced lung volume, and increased collapsibility of the upper airway (Series *et al.* 1990; Owens *et al.* 2010). These changes are likely to contribute to the higher prevalence of OSA among the obese (Gami *et al.* 2003). The obese volunteers in this study tended to have narrower airways than leaner subjects with larger airway dilatation during inspiration. As the relationship between obesity and airway size was strong, it was not possible to include both variables in the multiple regression due to collinearity. Therefore, it is difficult to split the roles of obesity and airway size in airway dilatation during inspiration from our data. While

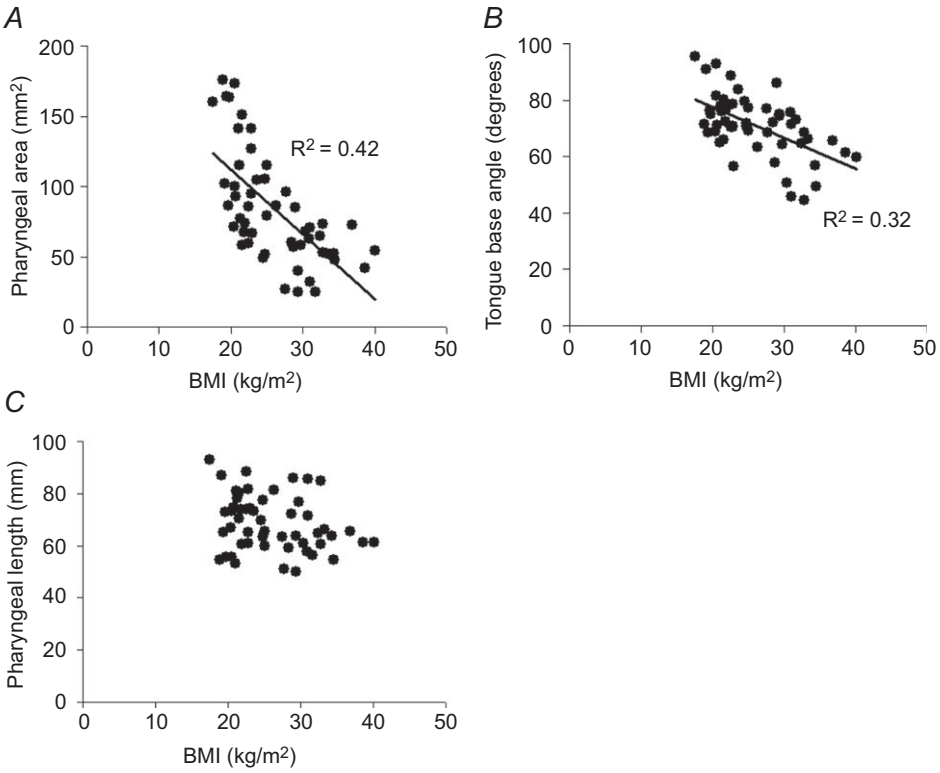

Figure 4. Relationships between BMI and upper airway variables
Relationship between BMI and pharyngeal cross-sectional area (*A*), tongue-base angle (*B*), and pharyngeal length (*C*), for all subjects. In this and subsequent figures data from each subject are shown as filled circles ($n = 52$). Linear relationships between BMI and pharyngeal area and tongue-base angle are significant ($P < 0.00001$ for both) but the relationship between BMI and pharyngeal length is not ($P > 0.05$). R^2 is the coefficient of determination for the linear regression lines shown.

our sample did not include sufficient numbers of females with a high BMI to rule out sex differences at a high BMI, the airway motions appeared similar for males and females with a similar BMI.

The effects of ageing on airway physiology are thought to be similar in normal men and women (e.g. Rowley *et al.* 2001), although one study reported a larger age-related decrease in the EMG response of genioglossus to negative pressure in men than women (Malhotra *et al.* 2006). Anatomically, the airway becomes more circular with age and the airway length has been reported to increase in women but not men (Malhotra *et al.* 2006). The latter is in contrast to the current study, which found that the pharynx lengthened modestly in the group as a whole. However, there was no significant relationship between pharyngeal length and dilatory motion or sex. There is also some suggestion of skeletal changes (increased retrognathia) associated with ageing (Malhotra *et al.* 2006) in which antero-posterior bony dimensions may be reduced by remodelling. This could contribute to a

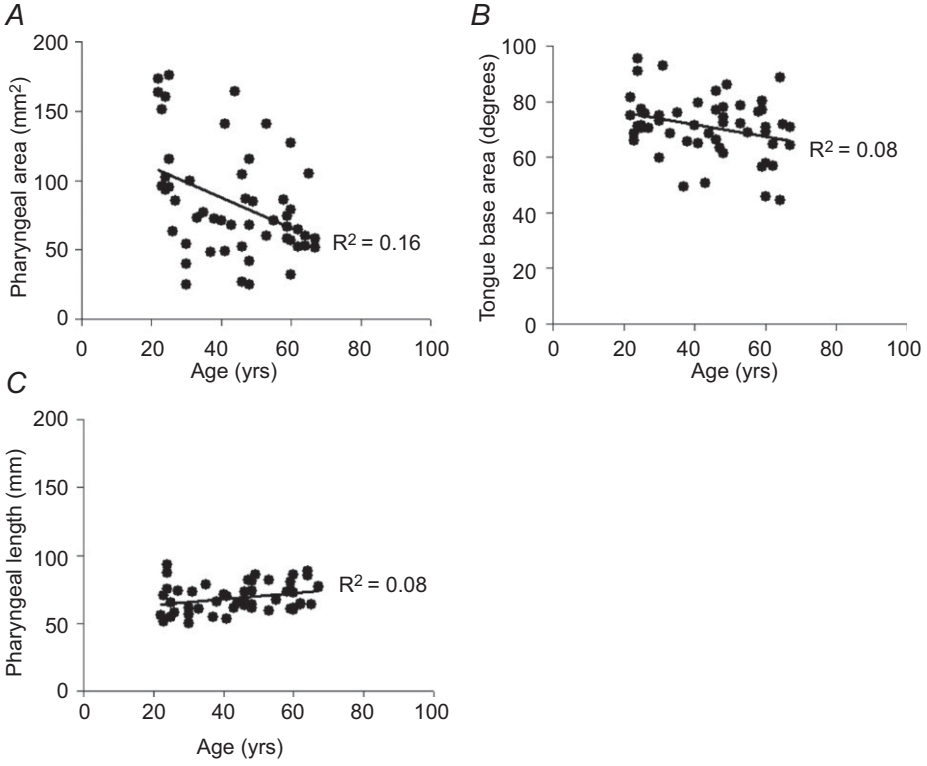

Figure 5. Relationships between age and upper airway variables
Relationship between age and pharyngeal cross-sectional area (*A*), tongue-base angle (*B*), and pharyngeal length (*C*), across all subjects. All linear relationships are significant ($P = 0.003$, $P = 0.036$, $P = 0.038$, respectively).

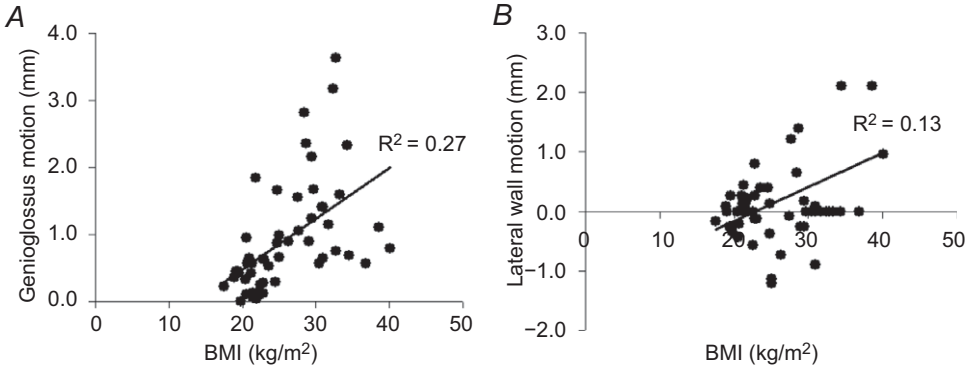

Figure 6. Relationships between BMI and movement of genioglossus and the upper airway
Relationship between BMI and movement of genioglossus (anterior movement is defined as positive, $P < 0.0001$) (*A*), and mediolateral movement of the lateral airway wall tissue (lateral movement is defined as positive, $P = 0.009$) (*B*).

narrower airway in older subjects. The effect of age on upper airway collapsibility is not clear, as some studies suggest there is an increase in collapsibility with age independent of obesity (P_{close} increases up to age 45 years) (Eikermann *et al.* 2007), while others show that age is not associated with changes in airway collapsibility in men, but is associated with increased collapsibility in women after adjustments for BMI (Kirkness *et al.* 2008). In the current study, among healthy subjects across a broad range of ages and body mass indices, age was weakly correlated to airway dilatation in univariate correlations, but this relationship was not significant in the multiple

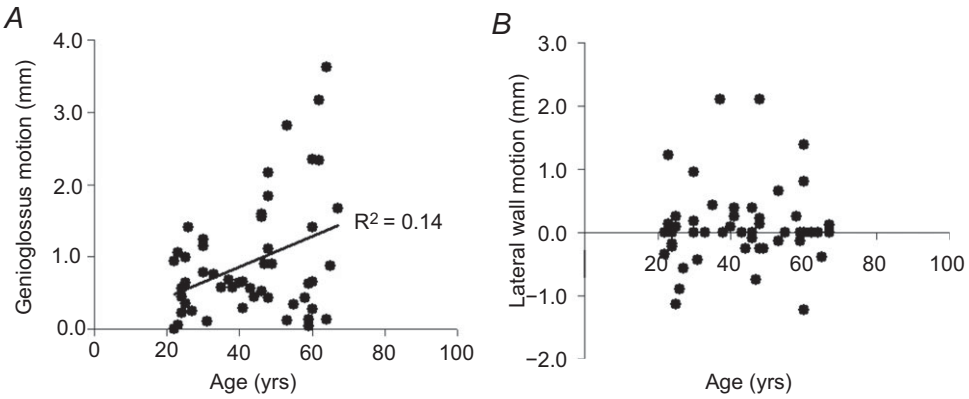

Figure 7. Relationships between age and movement of genioglossus and the lateral airway
Relationship between age and movement of genioglossus (anterior movement is positive, $P = 0.006$) (*A*), and mediolateral movement of the lateral airway wall tissue (lateral movement is defined as positive, $P = 0.22$) (*B*). The relationship between age and genioglossus motion is significant, but not that between age and lateral wall motion.

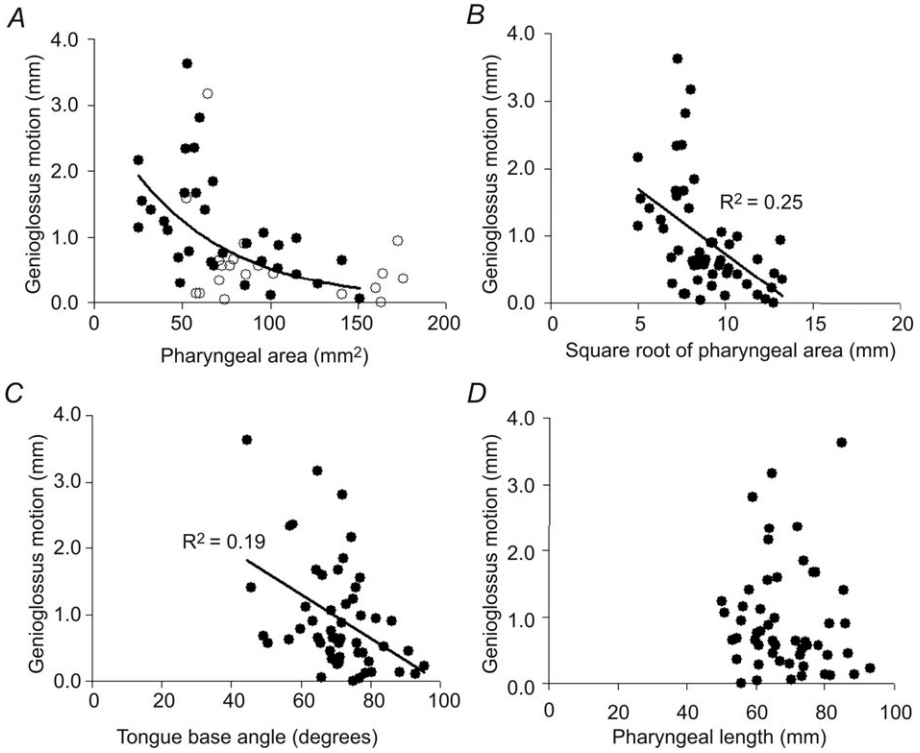

Figure 8. Relationships between pharyngeal area, tongue-base angle and pharyngeal length, and anterior motion of genioglossus
Relationship between anterior motion of genioglossus and pharyngeal area (*A*), square root of pharyngeal area ($P = 0.0002$) (*B*), tongue-base angle ($P = 0.001$) (*C*), and pharyngeal length ($P = 0.7$) (*D*). Open and filled circles in *A* denote females and males, respectively. All relationships are statistically significant except that between genioglossus and pharyngeal length.

regression model. This suggests that in healthy adults within the ages studied here (22–68 years), age is at most a minor contributor to changes in active airway dilatation.

In contrast to the active dilatation observed during inspiration in healthy subjects with small airways during wakefulness, many OSA patients with equally narrow airways do not actively dilate their airway during inspiration while other patients have bi-directional motion of the airway walls, where one region dilates but another narrows (Brown *et al.* 2013). The latter pattern was rarely observed in healthy volunteers in this study. The lack of dilatory motion in patients is unlikely to be due to inadequate neural drive, as drive is thought to be higher during wakefulness in OSA patients (Malhotra *et al.* 2002) or at least not reduced based on single motor unit recordings (Saboisky *et al.* 2007; Saboisky *et al.* 2012). The neuropathy revealed by the latter studies may reduce the effectiveness of neural drive in shortening genioglossus muscle fibres. Interestingly, during propofol anaesthesia, increases in genioglossus EMG produced by prolonged hypopnoeas did not increase airflow or pharyngeal cross-sectional area. This suggests that neural drive to the upper airway can be dissociated from effective active dilatation of the upper airway in OSA patients (Dotan *et al.* 2013). There is another relevant mechanical factor in OSA patients. Because their tongues are commonly enlarged (Chi *et al.* 2011), there is less 'space' in the oral cavity for the displaced tissue, and this would restrict tongue motion. Some of this volume change results in chronic postero-inferior displacement of the tongue and a reduction of the tongue-base angle (see Chi *et al.* 2011). As the tongue acts as a muscular hydrostat (Kier & Smith, 1985), tongue volume is constant, so if it moves anteriorly in one region near the airway wall, the same volume of tissue must be displaced elsewhere. To test such a mechanism would require high-resolution dynamic volumetric measurements of the motion of the whole genioglossus within the oral cavity. This is technically challenging with current imaging techniques. Further studies are also needed to see if the movement patterns observed here during wakefulness persist during sleep, and whether the differences observed in movement patterns of healthy subjects compared to OSA patients (Brown *et al.* 2013) underpin the nocturnal airway collapse. It would also be interesting to determine whether weight loss or gain could alter an individual's airway dilatory response to maintain patency, both in healthy subjects, and in OSA patients.

If the largely normal level of neural drive delivered to the genioglossus in OSA patients with narrow airways does not result in the dilatation observed here in healthy controls, it could also 'stiffen' the genioglossus. However, this effect, if present, is not sufficient to restore the stiffness of the tongue to that of matched healthy subjects. This has been established using MR elastography to quantify the shear modulus of the tongue (Nye *et al.* 2011).

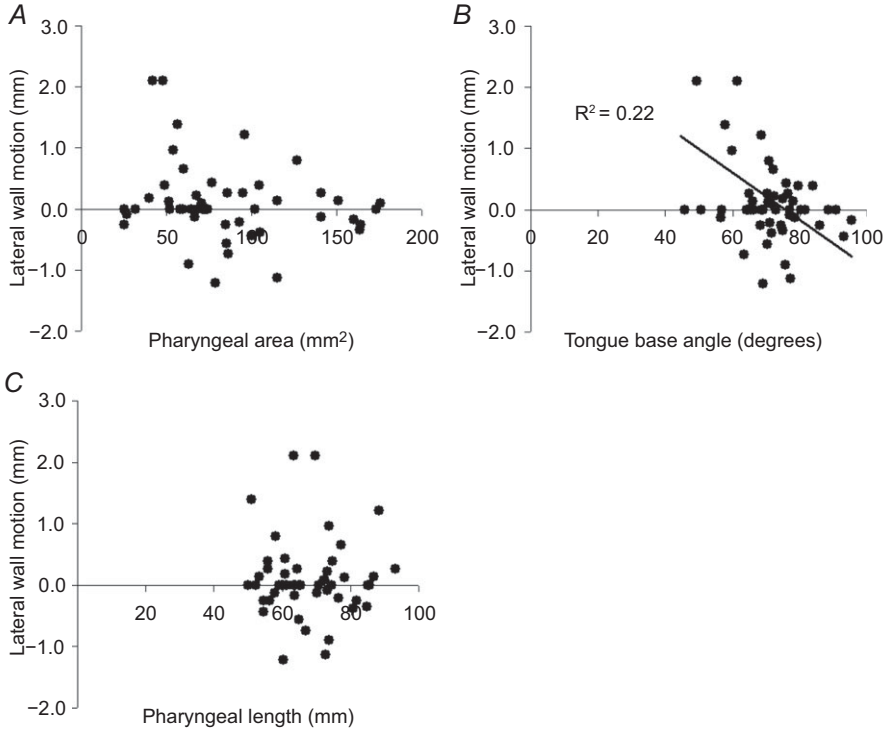

Figure 9. Relationships between lateral wall motion and anatomical parameters
The relationship between lateral motion of the upper airway and pharyngeal area ($P = 0.11$) (*A*), tongue-base angle ($P = 0.0005$) (*B*), and pharyngeal length ($P = 0.93$) (*C*). Only the relationship between lateral wall motion and tongue-base angle is significant.

In the present study, subjects were imaged while awake as it is difficult to sleep in MRI scanners due to the loud noise without sedative medication which is likely to change upper airway function. This limitation means that it is not yet clear if the relationship between airway size and dilatation during inspiration operates in the same way during sleep, or if sleep state-related changes in neural drive to the upper airway dilators influence this relationship. The analysis here is based on tracking the motion of specific points in the genioglossus and lateral airway wall. Qualitatively, these points represent 'typical' responses within the regions studied and were chosen based on previous studies of airway tissue motion (Cheng *et al.* 2008; Brown *et al.* 2013). However, variations in airway dilatation, or in the shape of the airway along the length of the pharynx, were not quantitatively analysed in this study. The imaging technique used here is also inherently a 2-D technique, precluding detailed 3-D analyses of motion. High spatial and temporal resolution 3-D imaging techniques that are amenable to quantification of soft tissue motions during respiration are not yet widely available. The pharyngeal length was measured in the vertical imaging axis, which could introduce small errors if the pharynx is not aligned with the image plane. In our data, the pharynx angulation, quantified as the angle of the posterior pharyngeal wall, was less than 10 deg for all subjects, which would result in a less than 2% error in length measurements, so this is unlikely to alter our conclusions about the influence of pharyngeal length.

Our findings provide improved understanding of the control of the upper airway in healthy subjects. We have identified a new relationship between airway size and the degree of active dilatation required to maintain upper airway patency during inspiration in wakefulness. In healthy subjects with a narrow airway, the upper airway dilates actively during inspiration to facilitate airflow, while subjects with larger airways dilate the airway less. In contrast, under similar conditions, previous studies have shown that OSA patients tend not to dilate the airway.

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Note added in proof: A recent study of overweight and obese subjects without obstructive sleep apnoea examined their response to reductions in airway pressure during sleep (not rapid eye movement sleep) (Sands et al 2014). These subjects had a more collapsible airway and increased EMG activation of genioglossus than normal-weight subjects. Sands SA, Eckert DJ, Jordan AS, Edwards BA, Owens RL, Butler JP, Schwab RJ, Loring SH, Malhotra A, White DP & Wellman A (2014). Enhanced upper-airway muscle responsiveness is a distinct feature of overweight/obese individuals without sleep apnea. American Journal of Respiratory and Critical Care Medicine (in press).

Additional information

Competing interests

The authors have no competing interests to declare.

Author contributions

All experiments were carried out at the NeuRA Imaging Centre, Neuroscience Research Australia. All authors except A.H. contributed to the design of the study and interpretation of the data. The article was drafted by S.C., L.E.B., S.C.G. and E.C.B., and revised by A.H. and J.E.B. for important intellectual content. S.C., A.H. and E.C.B. collected the data and S.C., L.E.B. and A.H. analysed the data. All authors approved the final version of the manuscript.

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