



Original Article

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Characteristics of Obstructive Sleep Apnea in Obese Minipigs

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Abstract

The report comprehensively evaluated physiologic, anatomic, tissue, functional, and modeled air flow characteristics of obstructive sleep apnea (OSA) in four obese and three non-obese control minipigs (8.5-months to 6.5-years. The approaches included: 1) Sleep monitoring to identify OSA during natural and sedated sleep; 2) Sonometric technique to quantify 3D respiratory deformational changes of the tongue base (TB) and soft palate (SF); 3) Fat-weighted MRI and ultrasound elastography (EUS) to calculate percentage of fat composition and tissue strain in TB, SF, and pharyngeal wall; 4) Sleep videofluoroscopy (SVF) to trace respiratory movements of SF and TB; 5) MRI to quantify 2D and 3D oropharyngeal airway dimensions and volumes along with respiratory airflow measurements; and 6) Computational flow dynamic (CFD) to model airflow dynamics. Results indicated: 1) The respiratory parameters of sedated sleep were similar to those of natural sleep, and the majority of OSA episodes occurred during rapid-eye-movement stage in obese minipigs; 2) Significantly extended respiratory deformations of TB and SF were seen in obeseOSA minipigs; 3) The fat deposition of TB increased from rostral to caudal and reached the highest in TB. Higher TB stiffness was seen in obeseOSA minipigs; 4) Larger ranges of respiratory movements of TB and SF were seen in obeseOSA minipigs; 5) Lower inspiratory tidal volume and slow inspiratory airflow speed were seen with decreased airway dimensions and volumes in obeseOSA minipigs; and 6) CFD confirmed that the decreased airflow speed occurred in the transitional region of nasal to oral pharynx. Therefore, OSA in obese minipigs presents similar characteristics to those of human, making obese minipig an ideal large animal model for OSA studies which would inform future human clinical trials.

Keywords

Obstructive sleep apnea, Obesity, Tongue base, Soft palate, Minipig

Introduction

Obstructive Sleep Apnea (OSA) is a common chronic sleep-related breathing disorder and affects more than 20% of the population with significant morbidity and mortality, and its prevalence is increasing as obesity reaches epidemic proportions in the US and worldwide. OSA is characterized by episodes of a complete (apnea) or partial (hypopnea) collapse of the upper airway with an associated decrease in oxygen saturation or arousal from sleep [1]. However, the pathophysiology and underlying mechanisms of OSA are poorly understood. While the US Congress “commends the Institutes (NIH) for its work on OSA and encourages the Institute to include surgical treatment in its work to define useful treatment for OSA” [2,3], the efficacy of various treatments including current mechanical and surgical applications, and experimental neuromuscular stimulation and pharmacological interventions are in debate, and resulted in mixed treatment outcomes. The studies in OSA mechanisms

and effective treatment approaches are greatly limited by significant constraints in examining *in vivo* oropharyngeal morphology and function in human subjects. Therefore, a validated and characterized animal model for OSA study is an imperative need. Although OSA can be induced in various animals including monkeys [4], minipigs [5], dogs [6], rabbits [7,8], cats [9], rats [10,11], and mice [12,13], only naturally

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Table 1: Physical characteristics of minipigs.

Pig No.	Age	Sex	BW (Kg)	BL (cm)	BMI (Kg/m ²)	NC (cm)
Obese Yucatan						
930	8.5 M	M	68	115	51.42	82
954	8.5 M	F	71	119	50.13	77
Obese Panepinto						
7	6.5 Y	M	85.6	132	48.75	82
10	6.5 Y	M	104.1	132	59.30	94
Non-obese Yucatan						
716	8.0 M	F	45.9	110	37.93	61
970	9.5 M	M	55	120	38.19	66
918	11.0 M	F	55.1	119	38.91	74

BW: Body Weight; BL: Body Length; measured from the tip of snout to the base of tail; BMI: Body Mass Index, calculated at body weight (Kg)/body length(m)².; NC: Neck Circumference, measured at the location of thyroid cartilage.

occurring OSA is a suitable animal model for studying OSA mechanisms and direct effects of treatment (beneficial and adverse). English Bulldogs have naturally occurring OSA, but it is caused by severely deformed upper airway and craniofacial structures, not the typical problem in human OSA. The other option is the obese Minipigs found in late 1990', but their OSA is not well validated or characterized [14,15]. Recently, a human imaging research indicated that obesity in OSA is associated with greater fat deposition in the tongue base compared to BMI matched controls [16]. This compelling finding may suggest possible new treatment approach aimed at reducing tongue fat. However, such invasive and irreversible therapy should be tested and optimized in an OSA animal model first, as is true of other innovative invasive therapies. Thus, a validated and characterized animal model with obesity-related naturally occurring OSA is of critical value for today's OSA study. The present study was to 1) Validate naturally occurring OSA in obese Minipigs; and 2) If OSA validated, characterize a) Respiratory internal kinematics of the tongue base and soft palate by the implanted ultrasound crystals; b) Fat composition in the tongue base, soft palate, and pharyngeal wall by fat-weighted MRI; c) Live tissue stiffness of the tongue base by ultrasound elastography; d) Respiratory dynamics and spatial relationships of oropharyngeal structures by live X-ray fluoroscopy, and e) Respiratory airflow parameters and computational airflow dynamics (CFD). The outcomes provided clues for the roles of the tongue base, soft palate, and pharyngeal wall in causing airway patency or collapse, and the mechanisms of how obesity confers risk for OSA, which would inform future human clinical trials.

Materials and Methods

Animals

The study included 7 minipigs, 3 non-obese control and 4 obese minipigs (Premier BioSource. CA) (Table 1). summarizes the physical features of these minipigs. Of the 7 minipigs, 5 were 7-8 month-old Yucatan minipigs with 3 normal controls and 2 obese, plus 2 retired Panepinto minipig aged 6.5 to 7-years-old (Panepinto & Associate, Mason Ville,

CO). Panepinto minipig is a crossbreeding between Yucatan and Vietnamese minipig [17]. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Washington (Protocol# 3393-04).

Sleep monitoring - OSA verification

All Minipigs underwent live remote sleep monitoring sessions through the real-time recordings of either natural or sedated sleep for 3-4 hours via two sets of wireless BioRadio systems (Great Lakes NeuroTechnologies, Valley View, OH). The detailed procedures for monitoring of natural and sedated sleep and the verification of sleep apnea/hypopnea episodes were reported elsewhere [18].

Sonometrics-Respiratory deformational changes in the tongue base and soft palate

Under anesthesia, bilateral genioglossus (GG), styloglossus (SG), and thyrohyoideus (TH) were exposed via the submandibular incision. The pairs of 0.10 mm wire electromyographic (EMG) electrodes (California Fine Wire, Grover Beach, CA) were inserted into each of these 3 muscles using a 25G needle, and eight 2 mm B barbed ultrasound crystals (Sonometrics Co. London, Canada) were implanted into the tongue base to circumscribe a cubic region (3D) in the tongue base (Figure 1A), and four additional ultrasound crystals were implanted into the soft plate to circumscribe a rectangular shape (2D) in the soft palate (Figure 1B). Signals from EMG and ultrasound crystals were synchronized by digital-analog input and output. The detailed procedures for these implantations, and the data recordings/analyses were described elsewhere [19].

MRI - Fat compositions of oropharyngeal structures

MRI images (3T Philips Ingenia Quasar Dual 3.0T whole body scanner) with T1 weighted Volumetric Isotropic Turbo spin echo Acquisition (VISTA) and Dixon fat water separated sequences were taken when the minipig was placed in the prone position within the scanner bore (Figure 2A). The fat fraction map was obtained as the ratio of the fat image to the sum of the water and fat images. The three regions of interest

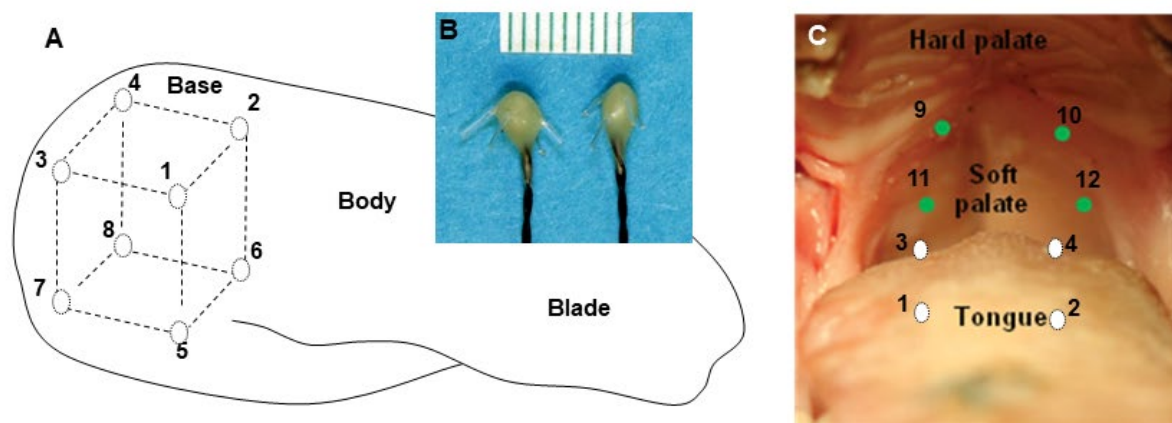


Figure 1: (A) Illustration of the configuration of 8 implanted ultrasound crystals in the tongue base to circumscribe a cubic region. Crystals #1 and #2 were implanted 2 mm posterior to the two circumvallate papillae and 3 mm underneath the dorsal mucosa; crystal #3 and #4 crystals were 20 mm posterior to the crystals #1 and #2 respectively and located in the dorsal area. Crystals #5, 6, 7, 8 were placed in the ventral region with a distance of 20 mm to crystals #1, 2, 3, 4, respectively; (B) 2 mm barbed ultrasound crystals; (C) Illustration of the 4 crystals (green dots #9-#12) implanted in the soft plate to circumscribe a square area. Note that white dots (#1-#4) indicate the 4 crystals implanted in the dorsal region of the tongue base. So, the change in distance changes among these two groups of crystals indicates the separation of the dorsal surface of the tongue base and ventral surface of the soft palate during respiration.

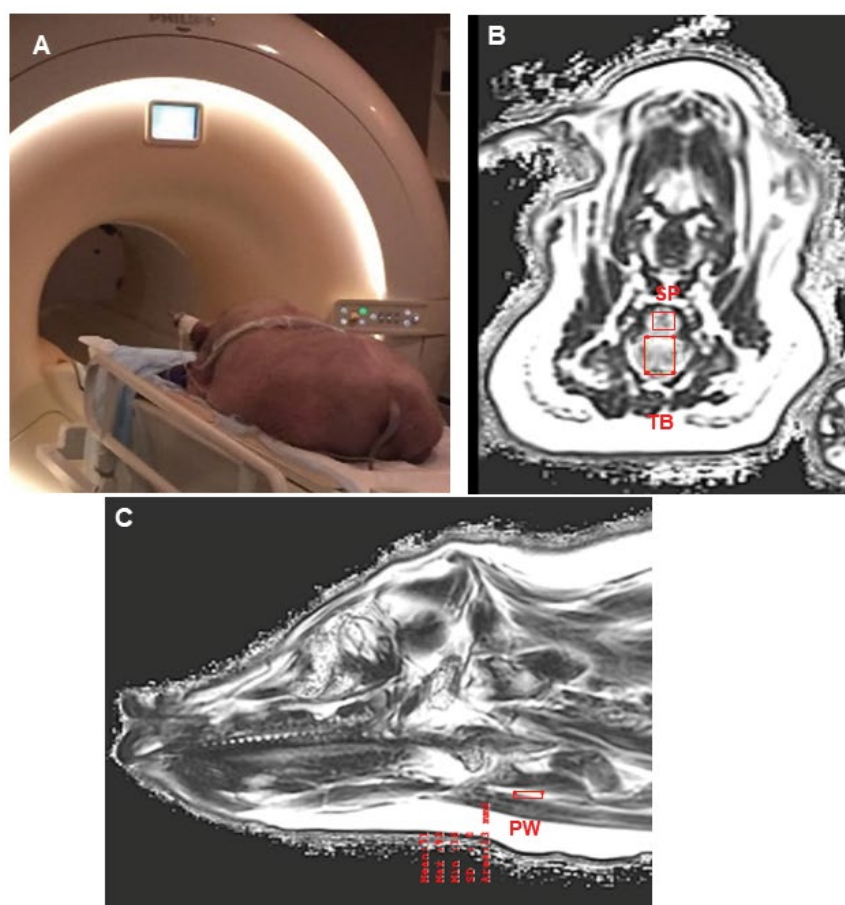


Figure 2: MRI imaging and Fat Composition measurements. (A) Live MRI scanning; (B) Coronal view of MRI Dixon fat water separated sequences. The two red boxes are the regions for the fat composition measurement of the tongue base (TB) and soft palate (SP). The measurements were taken in the serial 8 coronal slices from the most rostral to the most caudal with thickness of 5 mm in the tongue base and soft palate; (C) Sagittal view of MRI Dixon fat water separated sequences. The red box shows the region for the fat composition measurement of the pharyngeal wall (PW). The measurements were taken in the serial 3 sagittal slices from the mid-sagittal to the lateral and internal with thickness of 5 mm.

(ROI) are the tongue base, soft palate, and pharyngeal wall. The tongue base and the soft palate were analyzed using the coronal view (Figure 2B). For the pharyngeal wall, the mesial and distal borders were determined by establishing the first appearance and the last appearance of the pharyngeal wall in the sagittal view (Figure 2C). The total analyzed slices were 8 for the tongue base and soft palate, and 3 for the pharyngeal wall. The details about the analysis methods were described elsewhere [20].

Ultrasound elastography - *in vivo* tissue stiffness of the tongue base

Ultrasound elastography (USE) was collected with an Aixplorer scanner from Supersonic Imagine (Aix-en-Provence, France). A curvilinear scanhead with a frequency bandwidth of 1-6 MHz (SSI transducer XC6-1) was placed in the submandibular region with sagittal orientation in the regions of rostral, middle, and caudal tongue and at the left neck muscles when the pig was under sedated sleep in the lateral position. Each frame contained a colorized ROI in which the USE data were obtained by the instrument (Figure 2A); the color pixels of each ROI are related to the stiffness of the tissues. Custom-developed software automatically extracted a circular ROI from the center of the USE color box (Figure 2B). The diameter of the circular ROI was set equal to one-half of the average of the color box height and width (Figure 2C).

Additional custom MATLAB software mapped the image pixel colors to the USE values according to a color scale produced by the ultrasound scanner (Figure 2D). For each video clip the USE values in the extracted ROIs were averaged over the 5-10 frames, producing a single average circular elastography ROI. The USE pixel values were then averaged over the video clips acquired in each region of the tongue and the left neck muscles. More detailed information was reported elsewhere [20] (Figure 3).

Sleep video fluoroscopy - Respiratory dynamics of oropharyngeal structures

Sleep video fluoroscopy (SVF) was performed while the pig was under sedated sleep and placed on the C-arm table with lateral positions at 30 frames/s using a GE C-Arm video-fluoroscopy unit (General Electric Co. MA, (Figure 4A). Image J software (NIH, Bethesda, MD) was used to digitize SVF video clips. To define the respiratory movement direction and range of the tongue base and the soft palate, a reference coordinate was first created, and the dorsal surface of the tongue base and soft palate were outlined. The movements of the tip of soft palate and the dorsal surface of the tongue base were digitized and superimposed in every other video clip (sampling rate 15/s) for the two consecutive respiratory cycles in each animal (Figure 4B). For separating the respiratory phases, the transition from inspiratory to

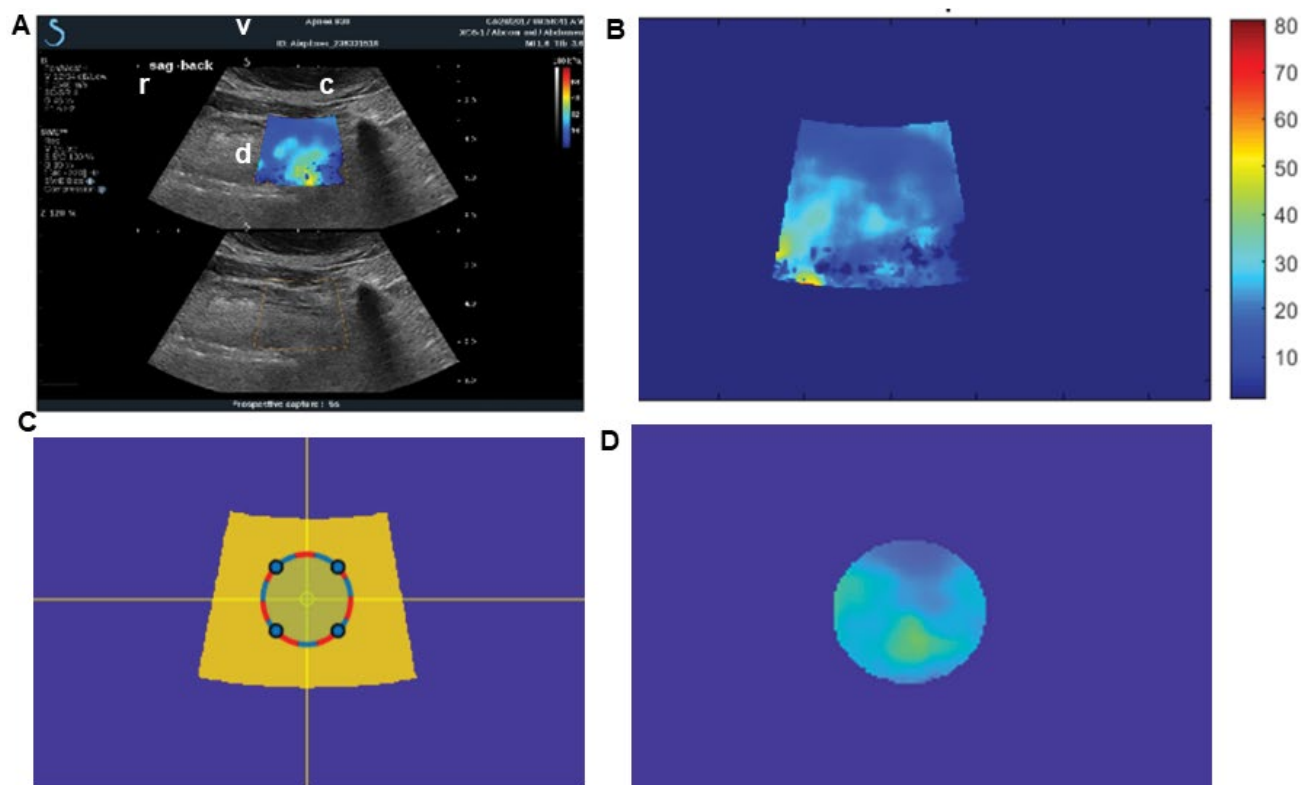


Figure 3: Ultrasound Elastography (USE). (A) Sagittal USE image of the tongue. The elastography measurement region is shown with color overlay in the top image. The dark region on the right side of the image is due to shadowing from the hyoid bone; (B) Colorized region of interest (ROI) with the scale bar converting color to stiffness value (kPa); (C) Circular ROI from the center of the USE color region; (D) The stiffness measurement according to the color scale in B for the extracted circular ROI. r: rostral, c: caudal; v: ventral; d: dorsal.

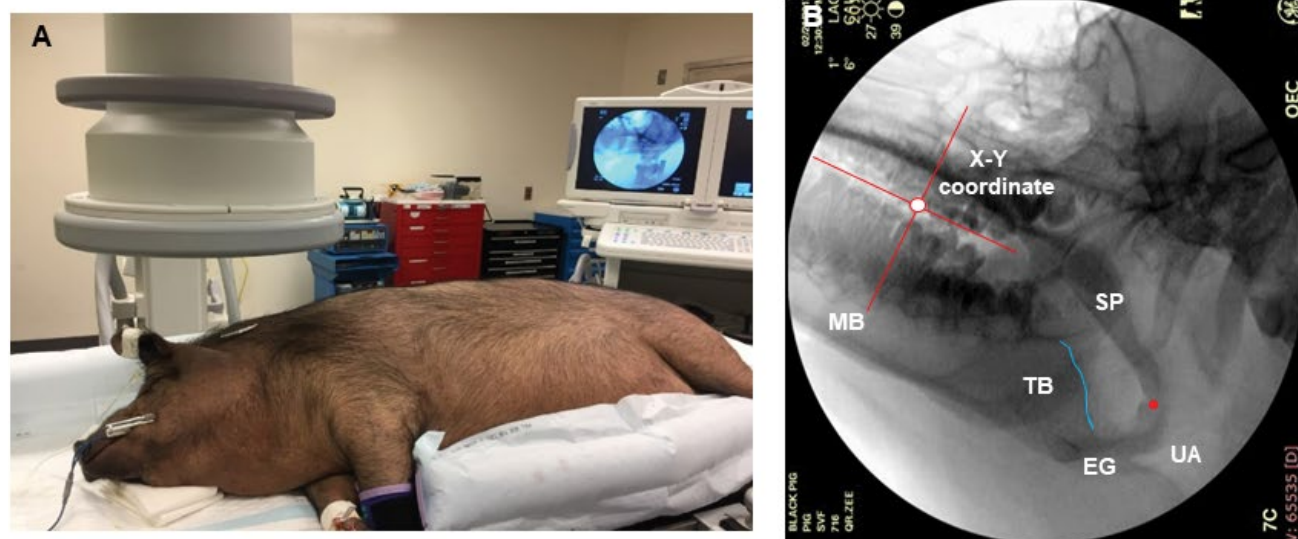


Figure 4: Sleep video-fluoroscopy (SVF). (A) SVF imaging; (B) Sagittal SVF image of oropharyngeal region; MB: Mandible; TB: Tongue Base; SP: Soft Palate; EG: Epiglottis; UA: Airway. The blue line indicates the dorsal surface of the tongue base, and the red dot indicates the tip of the soft palate. The red cross indicates the X-Y coordinate axes, and the white dot indicates the origin of the coordinates.

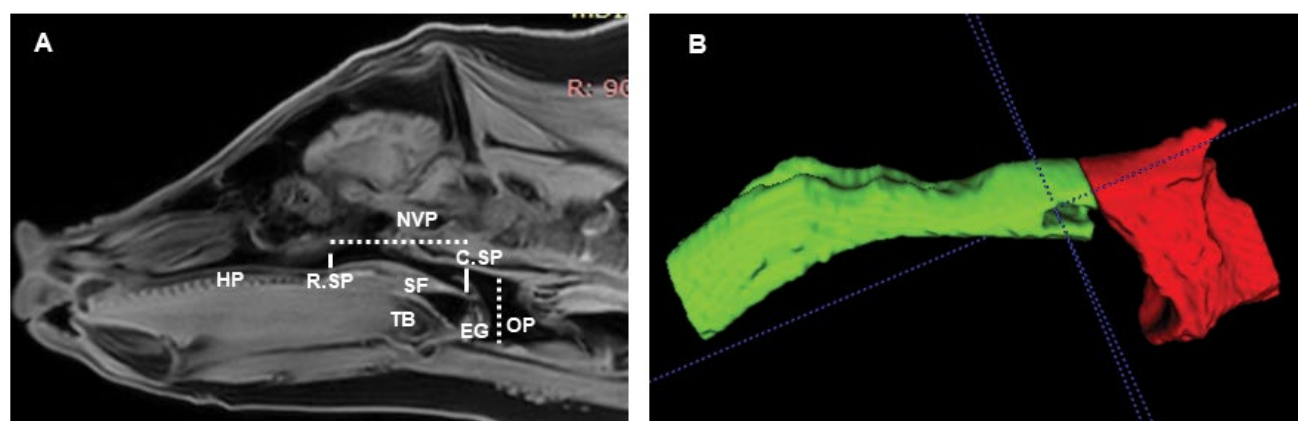


Figure 5: (A) VISTA MRI sagittal slice by RadiAnt DICOM Viewer to measure dimensions of nasal/velar and oral pharyngeal airway spaces. HP: Hard Palate; SF: Soft Palate; BT: Tongue Base; EG: Epiglottis. Two vertical lines indicate the rostral and caudal ends of soft palate (R.SP and C.SP). Horizontal and vertical dotted lines indicate the locations of nasal/velar and oral pharyngeal airway spaces (NVP and OP), respectively. (B) The segmented 3D model of the nasal/velar (green) and oral (red) pharyngeal airway spaces.

expiratory phases was determined by the video clip showing the maximal airway expansion, and usually the duration of inspiratory was shorter than the expiratory phases by the ratio of 0.65:1 [20].

Computational flow dynamics (CFD)

The images from the MRI Vista sequence with a resolution of 0.275 mm were used to create the 3D configurations of the nasal/velar and oral pharyngeal airway spaces. RadiAnt DICOM Viewer was used to measure the distance between the ventral and dorsal borders of the airway spaces between the rostral and caudal ends of the soft palate, and the beginning of the trachea caudal to the epiglottis. These measurements provided airway dimensions of the nasal/velar pharynx at the beginning and end of the soft palate, and the oropharynx

caudal to the soft palate and epiglottis (Figure 5A). Then, the ITK-Snap software merged the 2D MRI slices together to create 3D configurations of nasal/velar and oral pharyngeal airway spaces in the X, Y, and Z coordinates. The region of interest (ROI) was defined as the same as the airway dimension measurements: i.e., the rostral edge of the soft palate served as the anterior border, the beginning of the trachea served as the posterior border, the soft palate to the trachea as the ventral border, and the pharyngeal wall as the dorsal border. Within these boundaries, ITK-Snap was used to divide the volume of the ROI into 3D cubic voxel units. The volume within each voxel was integrated and then amalgamated together to form a 3D configuration of the airway spaces within the ROI. The ITK-Snap was further used to segment 3D configurations of the airways spaces by dividing them into the nasal/velar

(anterior) and oral (posterior) pharyngeal portions to isolate the location of possible turbulence in CFD modeling. These 3D configurations were used for CFD simulation (Figure 5B). The airflow parameters were collected using the Research Peumotach system (Hans Rudolph Inc. Shawnee, KS) through a facemask. The detailed CFD simulation and analysis were reported elsewhere [21].

Reliability tests and statistics

Both intra- and inter-reader reliability tests were performed for MRI and SVF measurements. The same investigator measured the same 10 MRI slices and 15 SVF video clips twice at 10 days apart, and the second investigator performed the same image analyses on these MRI frames and SVF video clips. Wilcoxon signed rank tests showed no significant differences between the two measurements for both MRI and SVF images for inter-reader tests ($p = 0.23$, r^2

$= 0.99$). The measurements of the intra-reader errors were calculated by Dahlberg's equation [22] and the error was 0.72%.

Due to the limited sample sizes, some of the data sets were not normally distributed. Therefore, both parametric ANOVA/non-paired T and nonparametric Kruskal-Wallis/Mann-Whitney tests (SPSS, IBM®) were used to examine the differences of each measured parameters between obese and control minipig groups with the significant level of $p < 0.05$.

Results

OSA verification

No central or mixed apnea was identified due to constant signals of respiratory movements from abdominal and chest belts during all recordings. The typical tracings in different stages of sleep are illustrated in Figure 6. Only one of the

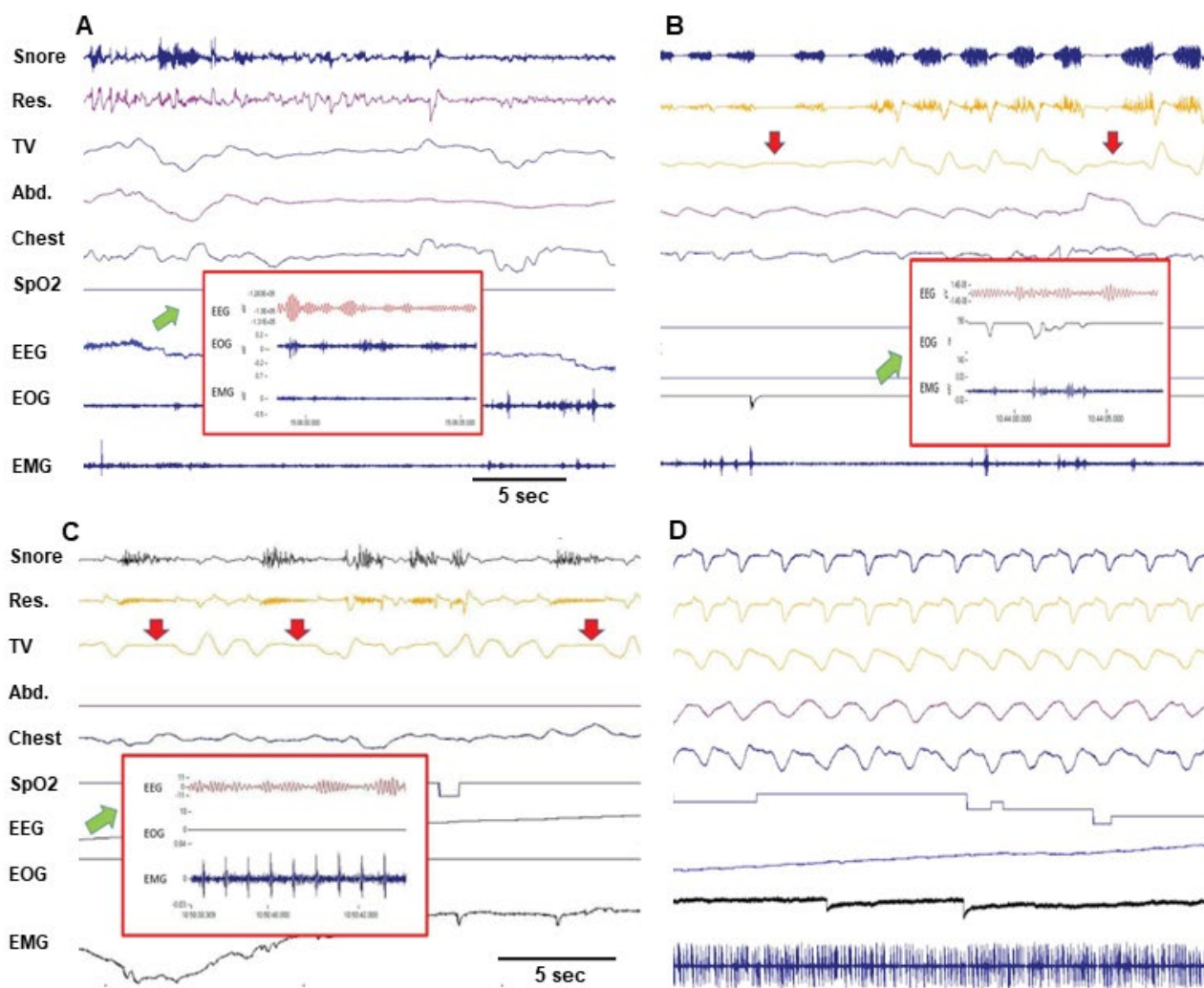


Figure 6: Raw tracings of physiological variables during natural and sedated sleep in the minipig #10. (A) Natural sleep (30 seconds); (B) Sedated sleep (30 seconds); (C) Respiration/snoring, tidal volume, and EEG during natural sleep (5 seconds); (D) respiration/snoring, tidal volume, and EEG during sedated sleep (5 seconds). Please note that C and D are not the zoomed tracings of A and B, but from the same recording session in the minipig #10. Res/Snore: Respiration/Snoring; TV: Tidal volume; Abd.: Abdominal movement; Chest: Chest movement; EEG: Electroencephalogram of the sites C3 and C4; EOG: Electrooculogram; EMG: Electromyogram of right pharyngeal middle constrictor. Please note that tracings of peripheral capillary oxygen saturation (SpO_2) are not listed, and the EEG in B is flat, because these recordings were not successful in this specific session.

Table 2: Comparison of AHI during natural and sedated sleeps.

Pig No.	Natural			Sedated		
	Total	NREM	REM	Total	NREM	REM
Obese Yucatan						
930	32	13	19	30	13	17
954	35	0	35	31	8	23
Obese Panepinto						
7	n/a	n/a	n/a	59	32	27
10	28	5	23	53	36	17
Non-obese Yucatan						
716	n/a	n/a	n/a	5	0	5
970	n/a	n/a	n/a	0	0	0
918	n/a	n/a	n/a	0	0	0

AHI: Apnea and Hypopnea episodes per sleep hour; NREM & REM: Non-Rapid & Rapid Eye Movement stages, respectively; n/a: not available.

3 controls experienced hypopnea episodes (AHI = 5). For sedated sleep, AHIs were significantly greater in all obese than control minipigs ($p < 0.05$) during either rapid and non-rapid eye movement (REM and NREM) stages, and the total and NERM AHIs were significantly higher in obese Panepinto than obese Yucatan minipigs ($p < 0.05$). In addition, AHIs were more often observed during REM than NERM stages during natural sleep in both obese Yucatan and Panepinto minipigs, but this was not the case during sedated sleep for older Panepinto minipigs (Table 2).

The ranges of oxygen saturation were wider in obese (85-100%) than control (93-100%) minipigs during both natural and sedated sleep, and there was no difference in the oxygen saturation between natural and sedated sleep. In obese minipigs, irregular breaths and absent/decreased airflow were more often seen in REM than NREM stages, followed by brief arousals. Interestingly, apnea and hypopnea episodes lasting 5-10 seconds were more often seen during sedated sleep, whereas those lasting longer than 10 seconds occurred more often during natural sleep.

Respiratory internal kinematics of the tongue base and soft palate

Corresponding to the respiratory rate, the stereotyped dimensional changes in the lengths, widths, and thicknesses of the tongue base along with the muscle activity bursts were observed (Figure 7A). Both dorsal and ventral lengths of the tongue base increased, while the widths of the tongue base increased dorsally and decreased ventrally, indicating elongation of both dorsal and ventral tongue base but widening in the dorsal and narrowing in the ventral tongue base during inspiratory phase. The thicknesses also showed opposite changes in the anterior and posterior tongue base, i.e., became thinner in the anterior and thicker in the posterior tongue base (Figure 7B). Therefore, the internal kinematic pattern of the tongue base during inspiration presented the following features: Elongation, dorsal widening and ventral narrowing, anterior thinning, and posterior thickening. During expiration, all lengths, widths, and thicknesses presented their dimensional changes in the opposite directions respectively.

Since respiration is a symmetric movement and no significant side differences were found as shown in Figure 7B, the values of both sides were averaged to reflect the overall changes in the dorsal/ventral lengths and anterior/posterior thicknesses. These results show that although the patterns of dimensional changes presented similar directions, their deformational ranges were different during respiration. While controls showed the smallest range from 0.05 to 0.23 mm, obeseOSA Yucatan minipigs exhibited 2 times larger ranges in elongation (0.14 to 0.19 mm) with similar ranges to the normal in the anterior thinning and posterior thickening, and smaller widening and narrowing ranges in the dorsal and ventral parts (0.06 to 0.11 mm), respectively. The two aged obeseOSA Panepinto minipigs presented significantly larger ranges with as much as 6-8 times more (0.71 to 1.24, $p < 0.05$) than controls in all lengths, widths, and thicknesses, possibly related to particularly heavy snoring during recording (Figure 8).

Due to the difficulty of access and the vulnerability of the crystals, data from the soft palate was limited. Similar stereotyped EMG bursts and dimensional changes of the soft palate were observed (Figure 9). In the phase of inspiration, the soft palate elongated symmetrically with larger and smaller widening in anterior and posterior regions, respectively in controls with the range of 0.02 to 0.05 mm. However, the ranges were more than 2 times (0.09 to 0.17 mm) and 4 times larger (0.32 to 0.81 mm, $p < 0.05$) in obeseOSA Yucatan Panepinto minipigs, respectively.

There were no directional alterations of the dimensional changes in the tongue base and soft palate between the control and obeseOSA minipigs, but the directional alteration between these two structures was seen. During inspiration, both anterior and posterior distances between the dorsal tongue base and soft palate were increased (0.09-0.12 mm) in controls, indicating the separation of the two structures. However, the anterior distance of the two in obeseOSA Yucatan and Panepinto/OSA minipigs was shortened (0.42-1.60 mm), indicating the possible closure between the two structures. The correlation analysis showed that neither

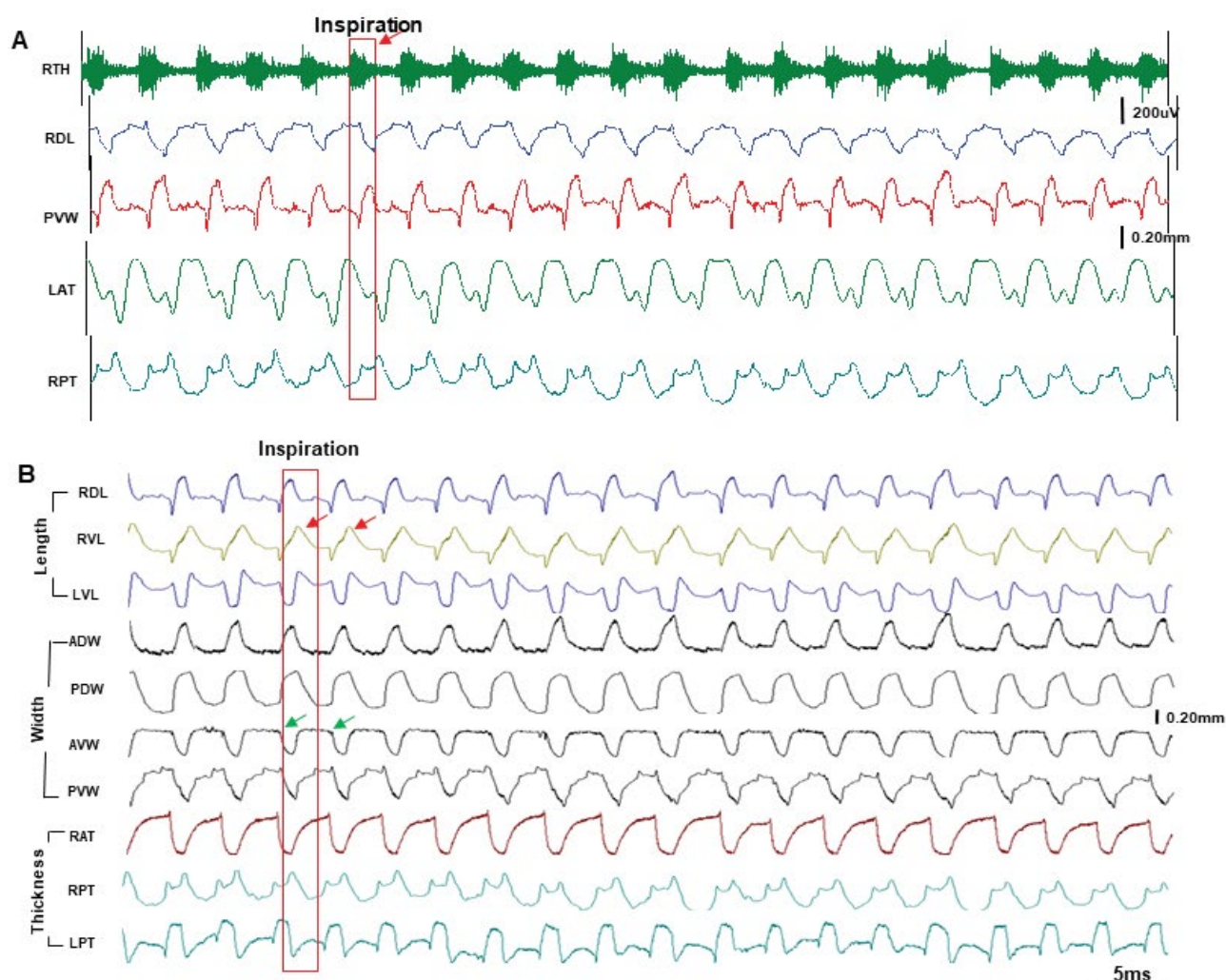


Figure 7: (A) Raw tracings of synchronized EMG and ultrasound crystals during respiration. Highlighted region indicates the inspiratory phase. RTH: Right Thyrohyoid Muscle; RDL: Right Dorsal Length; PVW: Posterior Ventral Width; LAT: Left Anterior Thickness; RPT: Right Posterior Thickness. Refer to Figure 1A for actual crystal pairs. The red arrow and highlighted region indicate the activity burst of RTH and inspiratory phase respectively; (B) Three dimensional raw tracings of ultrasound crystals in the length, width, and thickness of the tongue base during respiration. Each tracing indicates a given crystal pair. RDL: Right Dorsal Length; RVL and LVL: Right and Left Ventral Lengths; ADW and PDW: Anterior and Posterior Dorsal Widths; AVW and PVW: Anterior and Posterior Ventral Widths; RAT: Right Anterior Thickness; RPT and LPT: Right and Left Posterior Thickness. Red highlight indicates the inspiratory phase. The red and green arrows indicate distance increase and decrease of a given crystal pair respectively.

AHI nor BMI was associated with the ranges of respiratory dimensional changes in the two structures.

Fat composition in the tongue base, soft palate, and pharyngeal wall

The percentage of the fat composition in the tongue base was mapped against location to show a relationship between the distribution of fat composition from the anterior to posterior region of the tongue base. The boxplot indicates that there was gradually increased amount of fat composition from the rostral to caudal regions in all three groups, and the tongue base 30-40 mm caudal to the conjunction of the soft and hard palates had significantly higher fat composition than the region 5-15 mm rostral to the conjunction ($p < 0.05$, (Figure 10A). This trend was more significant in controls

and obeseOSA Yucatan than Panepinto OSA minipigs. Both Yucatan groups started at about 20% fat composition in the rostral tongue base and sharply increased to about 60-70% in the caudal tongue base. The trend of the obese Panepinto minipigs, however, showed less change in percentage of fat composition. It started at about 25% in the rostral tongue base and gradually increased to about 50% in the caudal tongue base.

For the percentage of fat composition in the soft palate, while the controls showed less changes from the rostral to caudal regions, both obese groups showed a downward trend from the rostral to caudal regions. Overall, lower fat composition was seen in Panepinto as compared with controls and obese Yucatan minipigs (Figure 10B).

The overall percentage of fat composition was significantly

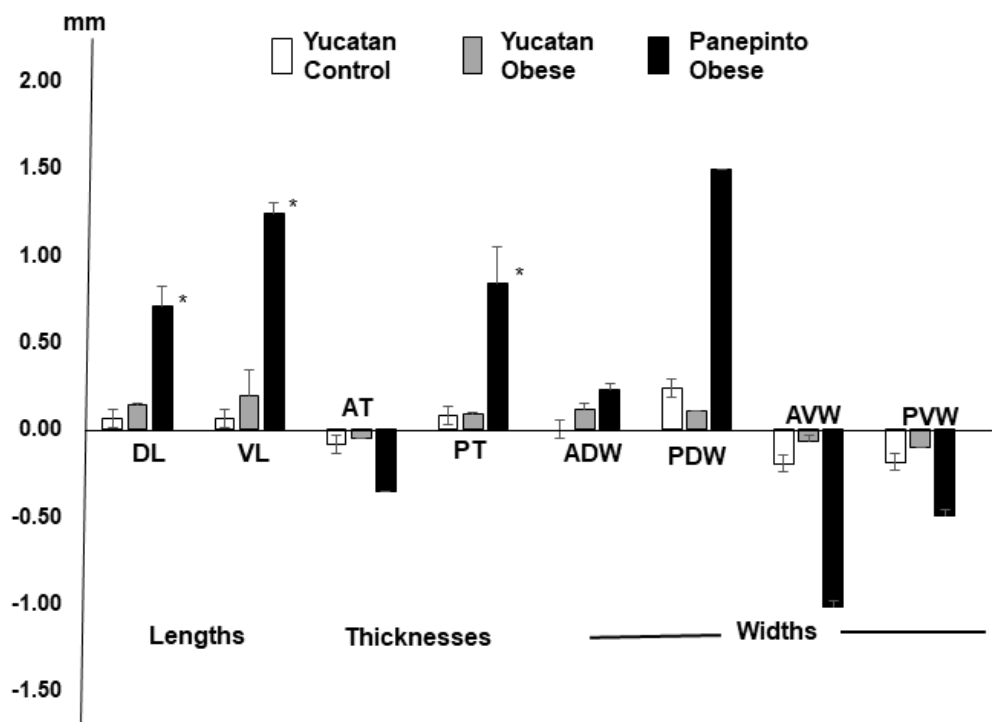


Figure 8: Bar graph showing inspiratory dimensional changes in dorsal/ventral lengths (DL and VL), anterior and posterior thicknesses (AT and PT), and anterior dorsal/ventral (ADW and AVW) and posterior dorsal/ventral (PDW and PVW) widths among three types of minipigs. *: indicate significant difference.

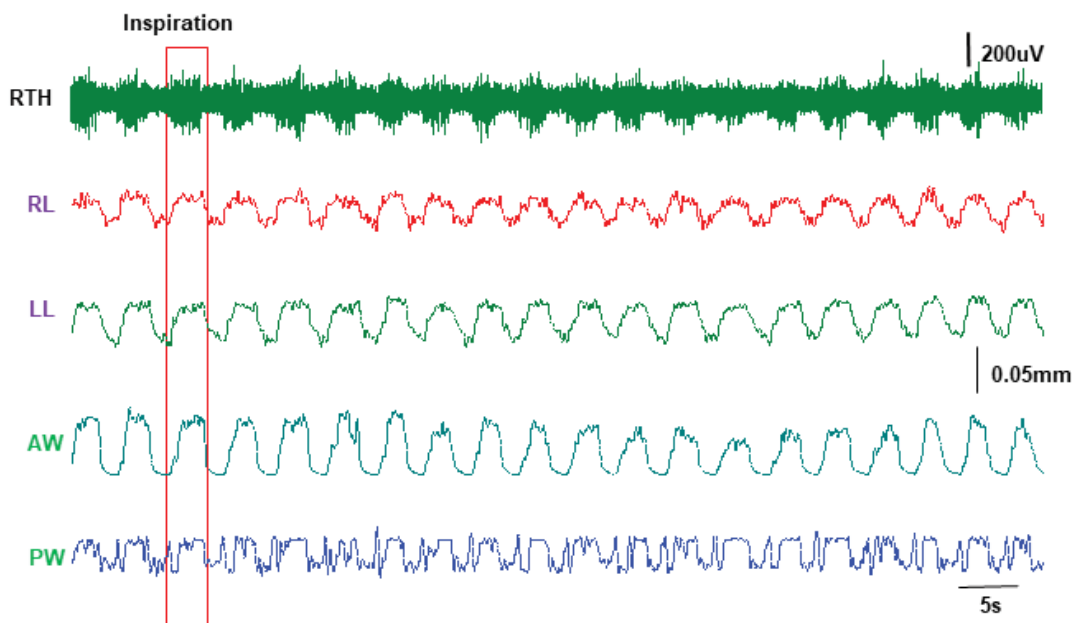


Figure 9: Raw tracing of synchronized EMG and ultrasound crystals in the soft palate. RTH: Right Thyrohyoid Muscle; RL and LL: Right and Left Length; AW and PW: Anterior and Posterior Widths. Highlighted region indicates inspiratory phase.

higher in the pharyngeal wall as compared with the overall values in the tongue base and soft palate, which was close to or even higher than the caudal tongue base and reached as high as 70-90%. Again, obeseOSA Panepinto presented (data from one animal only) a lower value at the region 5 mm lateral to the mid-sagittal region (Figure 10C).

Tissue stiffness of the tongue base

Overall, the obese minipigs presented higher stiffness values in all three regions of the tongue and neck muscles as compared to the controls, and the neck muscles presented the highest stiffness as compared to those of tongue regions

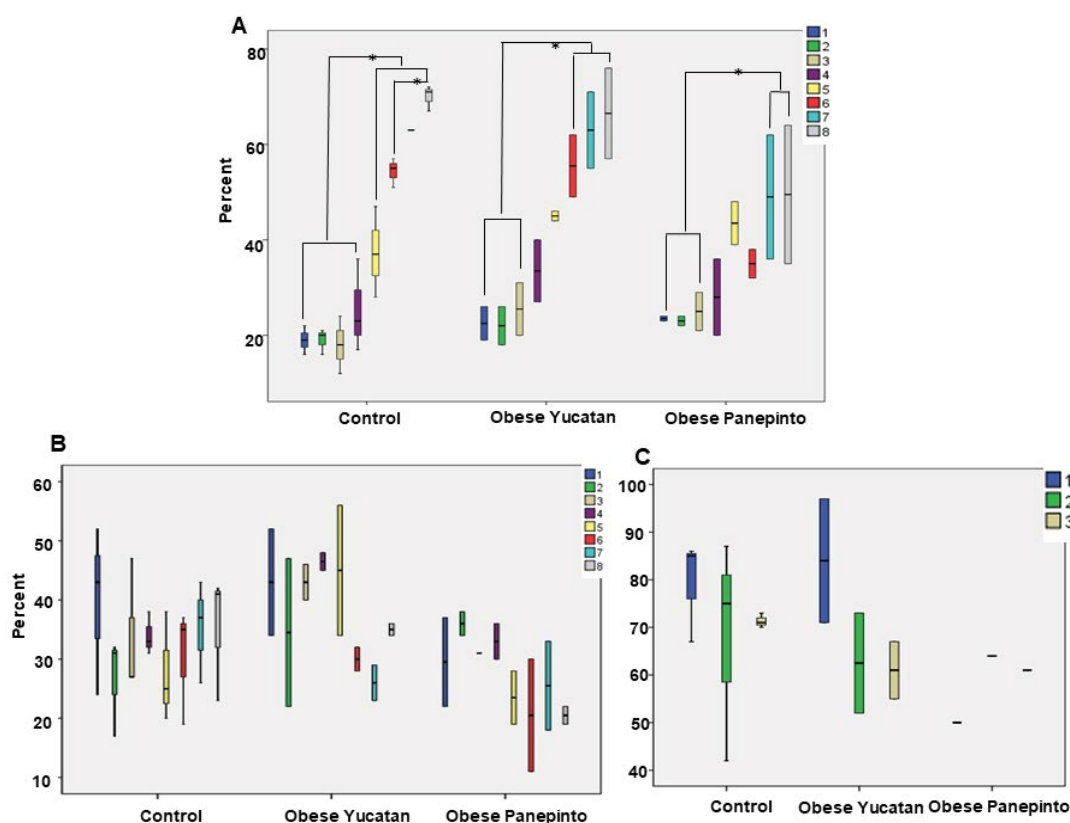


Figure 10: (A) Boxplots showing the % of fat composition from the rostral to caudal tongue base in each group. The color boxes and numbers indicate the coronal regions of the tongue base from the rostral to caudal regions. Numbers 1-8 indicate eight coronal regions from the most rostral (#1) to the most caudal (#8) region of the tongue base with an interval of 5 mm between regions. Asterisks indicate significant difference $p < 0.05$; (B) Boxplots showing the % of fat composition from the rostral to caudal soft palate in each group. Numbers 1-8 are the same as A but the soft palate. C. Boxplots showing the % of fat composition of the pharyngeal wall in each group. The color boxes and numbers indicate the sagittal regions of the pharyngeal wall from lateral, mid-sagittal and internal. Numbers 1-3 indicate 3 sagittal regions from lateral (#1), mid-sagittal (#2), and internal (#3).

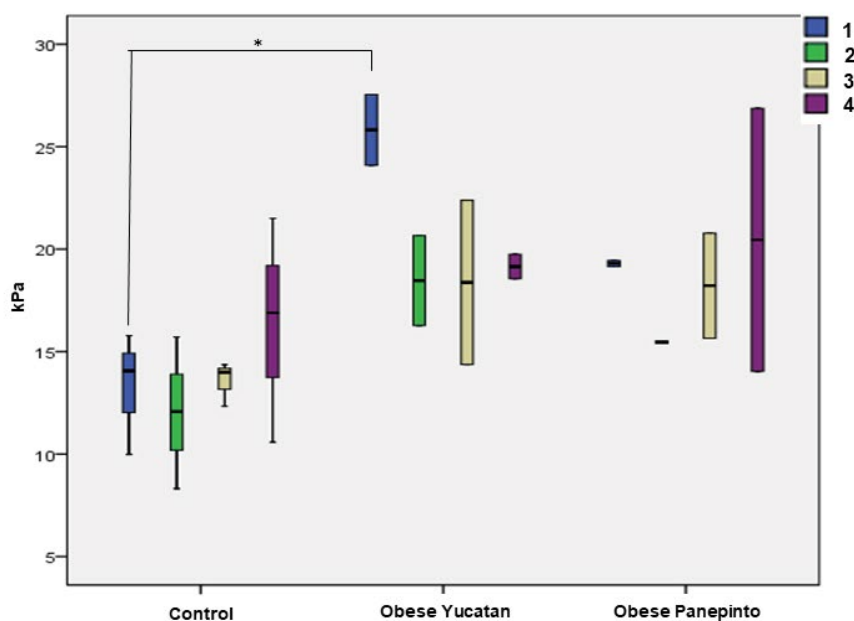


Figure 11: Boxplots showing the tissue stiffnesses of rostral, middle, and caudal tongue and the neck muscles by USE. The color boxes and numbers #1, #2, and #3 indicate rostral, middle, and caudal regions of the tongue, respectively, and #4 indicates neck muscles.

in each group, except the rostral tongue in Yucatan OSA obese minipigs. There were no obvious differences between the 3 tongue regions in each group except the rostral region in Yucatan obese minipigs, which was significantly higher than that in controls (Figure 11).

Respiratory movements of the tongue base and soft palate

The superimposed moving ranges of the dorsal surface of the tongue base were significantly smaller than these of the soft palate during respiration, and the component of its dorsal-ventral excursion (Y-axis) was much smaller than its rostral-caudal (X-axis) excursion (Figure 12). The respiratory moving range of the soft palate tip in both X and Y directions were small (2.33-3.67 mm) in controls, but significantly larger (4.02-7.50 mm) in obeseOSA Yucatan minipigs. In obeseOSA Panepinto minipigs, the moving range in X direction was similar to those in obeseOSA Yucatan minipigs, but the range in Y direction was highly significantly larger (30.38-31.68 mm) than those in other two groups (Table 3), which might contribute to significantly heavy snoring during expiration observed in both Panepinto minipigs during SVF imaging.

Morphology of oropharyngeal airway and CFD

Although much heavier body weights in obeseOSA minipigs, airway dimensions of these minipigs were either similar or smaller to those of controls, particularly obese #954, and the obese/OSA minipigs also showed smaller volumes in both nasal/velar and oral pharyngeal airway spaces (Table 4). Furthermore, the obeseOSA minipigs presented significantly lower tidal volumes of both inspiration (92.66 vs. 41.34 mL) and expiration (15.67 vs. 7.81 mL), and lower inspiratory airflow speed (14.15 vs. 7.81 LPM) compared to those of controls (p < 0.05, (Figure 13)).

Using the applied boundary conditions, the airflow velocity profiles along the dimensions of nasal/velar and oral pharyngeal airways were calculated for the respiratory phases in control and obeseOSA minipig models. No turbulence was observed in either model. The velocities generally varied with respect to the width of the airflow pathway where a narrower pathway correlated to a higher velocity. The velocity zero (V0) was designed as no slip conditions along the walls of the airflow pathway assumed, i.e., when the airflow first reached the wall of the pharyngeal airway. The velocity variations ranged from -15% to +10% of V0's in both inspiration and expiration for the control. In the obeseOSA minipig, the range was -10% to +25% of V0's, where the 25% increase was observed at the narrowest part of the airflow pathway located at the end of nasal/velar pharynx (caudal end of the soft palate), as indicated by the vertical arrows (Figure 14).

Table 3: Moving range of the soft palate tip (mm).

Groups	Pig #	X-axis	Y-axis
Controls	918	3.67	2.33
	716	2.64	3.24
	mean	3.16	2.79
Obese OSA Yucatans	930	4.02	6.39
	954	7.20	7.50
	mean	5.61*	6.945*
Obese OSA Panepintos	7	5.17	20.38
	10	8.92	31.68
	mean	7.045*	26.03**

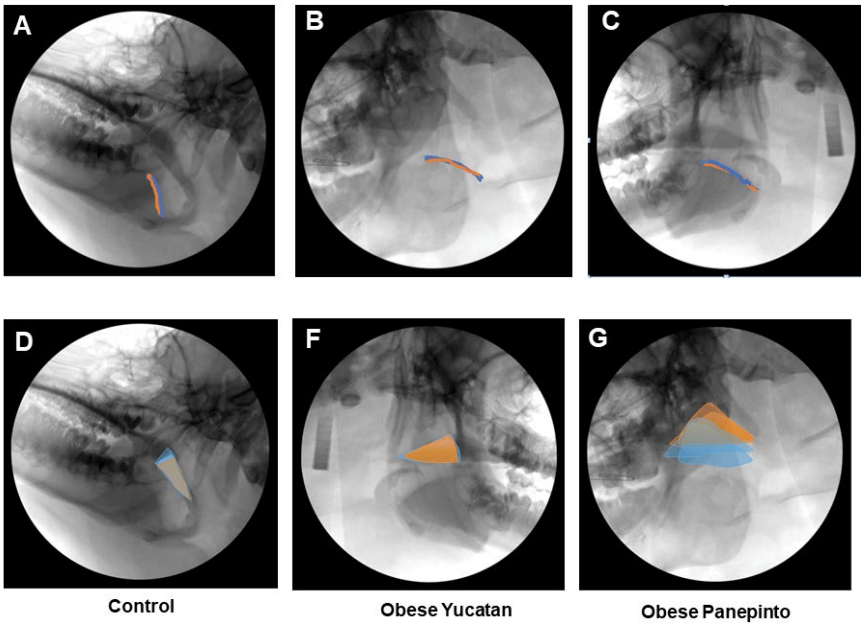


Figure 12: Superimposed moving trajectories of the dorsal surface of the tongue base (upper row) and tip of the soft palate (lower row) during inspiratory (blue) and expiratory (orange) phases during sedated sleep. All images were adjusted to have the lower border of the mandible parallel to the earth. A, D: non-obese Yucatan #716; B, E: Obese Yucatan #954; C, F: Obese Panepinto #10.

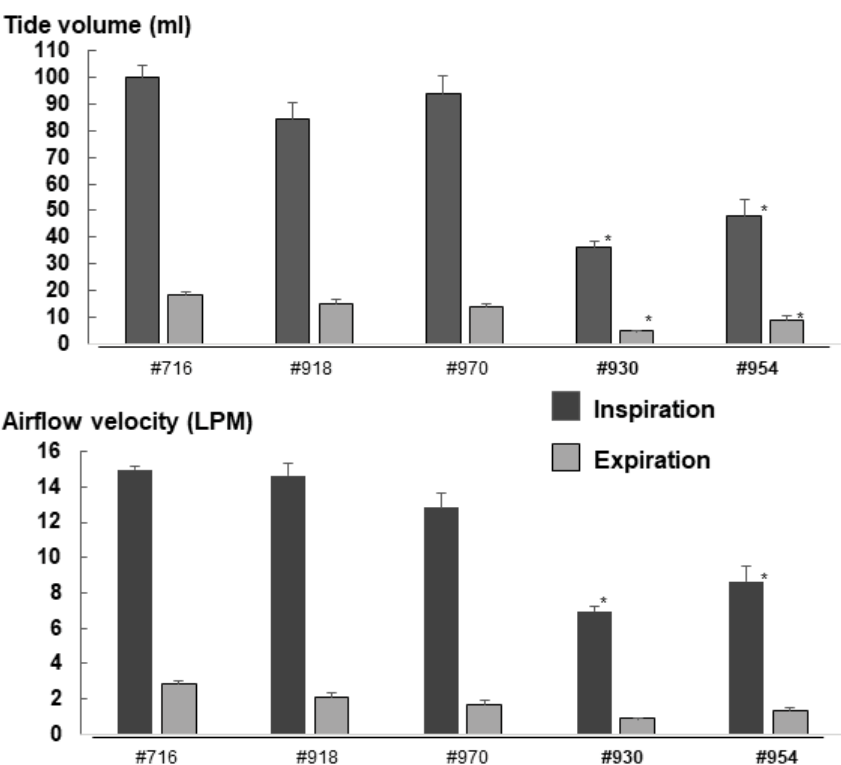


Figure 13: Comparisons of respiratory airflow parameters. (A) Tidal volume of inspiration and expiration (Milliliter, ml); (B) Airflow speed of inspiration and expiration (Liter Per Minute, LPM). **Bolded pigs' numbers:** obese OSA minipigs. Asterisks above the bars of obese animals indicate significantly lower values as compared to each of controls ($p < 0.05$).

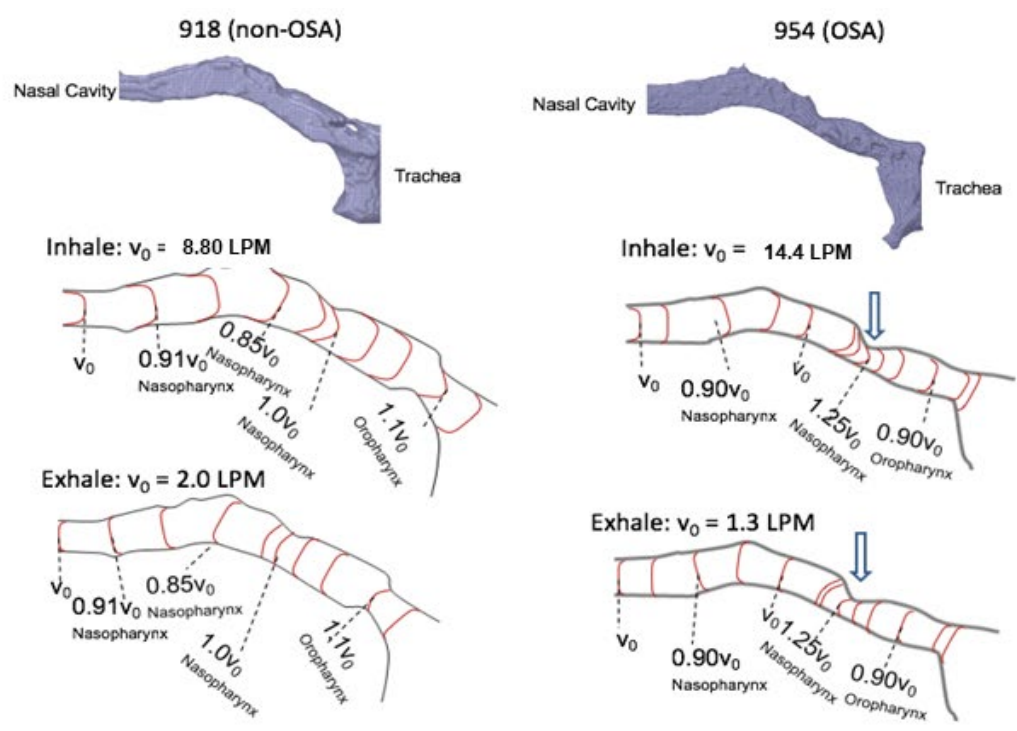


Figure 14: Comparisons of airflow velocity profiles along the nasal and oral pharyngeal airways between the representatives of control (#918, left column) and obese OSA (#954, right column) minipigs. (A) Nasal/velar and oral pharyngeal airways; (B) Airflow profiles during the inspiratory phase; (C) Airflow profiles during the expiratory phase. Please note that although turbulence is absent in airways of both CFD models, the airway of OSA obese minipig displayed an increased airflow speed at the narrowest portion of the nasopharynx (vertical arrows). LPM: Litter per Minute.

Table 4: Dimensional and volumetric measurements.

Pig #	Dimension (mm)			Volume (mm³)		
	R.SP	C.SP	OP	NVP	OP	Total
-						
716	10.17	8.46	29.84	10.10	8.68	18.78
918	8.89	16.18	37.05	13.91	16.64	30.55
mean	9.53	12.32	33.45	12.01	12.66	24.67
930	10.17	8.94	29.27	9.53	7.84	17.37
954	8.49	6.67	22.09	8.17	8.45	16.62
mean	9.33	7.81	25.68	8.85	8.15	17.00

R.SP: Rostral End of Soft Palate; C.SP: Caudal End of Soft Palate; NVP and OP: Nasal/Velar and Oral Pharyngeal Airways; 716 & 918: Controls; 930 & 954: Obese OSA Yucatan

Discussions

An appropriate animal model and OSA features

The pathogenesis and pathophysiology of OSA are poorly understood. Most importantly, the existing invasive treatment strategies have either critical limitations, unpredictable success, or unclear mechanism of effect. Conservative treatments, such as CPAP, oral appliances, physical therapy, and medications have either a low tolerance rate by patients or controversial outcomes. Therefore, there is an urgent need to establish a suitable large animal model for the study of OSA mechanism and testing of treatment strategies, including pharmaceutical therapy and adipose tissue reduction in the upper airway.

For years, several animal models have been used for OSA study. The minipig chosen in the present study is not only due to the similarities in the oropharyngeal airway regarding the architectures and tissue types, but also in individual shapes and size of oropharyngeal structures, such as the tongue, soft palate, and pharyngeal [23-26]. Most importantly, spontaneous OSA was identified in individual obese minipig [14] and was further validated and characterized in two different breed of obese minipigs in the present study [18,21]. In addition, most physiological instrumentation for monitoring OSA, surgical, electrical, and ultrasonic interventions for treating OSA, and pharmaceutical testing are only suitable for large animals with spontaneous OSA. Therefore, the obeseOSA minipig model has a great potential translational value as an ideal animal model for a variety of OSA mechanism studies and the evaluation of emerging treatment strategies for OSA.

Our choice to study obese minipigs was also based upon the following facts: 1) The upper airway is comparable to that of human, both showing a similar microstructural layered architecture composed of the same tissue types [26], and both are nasal breathers; 2) The tongue base strongly influences the upper airway through its contact with the soft palate [23]; 3) The time course of epiglottic movement is similar to that of human [27]; 4) The size and shape of the hyoid conform with those of human [28]; and 5) The minipig provides adequate size for instrumentation and therapeutic intervention. Despite these advantages, several differences in oropharyngeal airway between humans and pigs in anatomy

and physiology and limitations in sample sizes should be noted: 1) The respiration rate of the pig (25-30/min) [18] is about 100% faster than this in human (12-18/min) [29]; 2) Unlike humans, the pig has no descending upper airway, but the orientation of the upper airway in sleep positions is similar to that of human; and 3) The tips of soft palate and epiglottis are close to each other or even overlapped, but these two structures are separated widely in the vertical direction in humans. There are several limitations in the present study; 4) Sample sizes in each group are small, particularly lack of the same aged controls of Panepinto minipigs due to the source unavailability. Therefore, the observed differences in Panepinto obeseOSA minipigs could be derived from the different breeds of minipigs.

However, given the fact that the Panepinto is a Yucatan crossbreed [17], and obese Panepinto had similar BMI to obese Yucatan minipigs, it could be reasonably speculated that the observed differences between normal Yucatan and obeseOSA Panepinto minipigs were most likely resulted from obesity and/or OSA.

In the present study, most episodes of apnea or hypopnea lasted 5-10s, rather than longer than 10s as in humans. This fact confirmed that the actual length of apnea or hypopnea is proportional to the respiration rate. However, compared to Yucatan OSA minipigs, the aged and heavily weighted Penapinto OSA minipigs presented much lower respiratory rates and higher tidal volume during both natural and sedated sleep [18]. These facts indicate that, similar to humans, the respiratory rate decreases with age, and the tidal volume is closely related to the body size and weight.

In obese minipigs, more apnea or hypopnea episodes were found in REM than NREM stages during natural sleep, and the ratios of REM/NREM related apneas and hypopneas were about 2. This REM-predominant OSA mimics a common pattern seen in humans [30]. Even though the clinical significance of REM only OSA remains controversial, studies have shown that OSA-induced metabolic perturbation, including atherogenesis, hypertension, cardiovascular disease, and abnormal glucose metabolism, are more strongly related to obstructive respiratory events during REM than NREM sleep [30]. Interestingly, during sedated sleep, these REM-predominant apnea/hypopnea episodes were only seen in young obese and non-obese Yucatan, but not obvious in

older obese Panepinto minipigs. In addition, AHIs were much greater in these older Panepinto than young Yucatan minipigs (Table 2). These differences in OSA characteristics between the two minipig breeds may be related to their age and/or breed differences.

Sedated and natural sleep are the two states of unconsciousness with considerable physiological common ground [31]. Studies have shown that oral administration of sedation drugs has no effect on ventilatory response [32], and the confounding effect of light anesthesia on respiration is minor [33]. In addition, studies also found that EEG findings appear identical in sedated and natural sleep in horses [31], and patients with anatomically compromised airway tend to have obstructed airway in either state because depressant effects on muscle activation and ventilatory drive are shown in both states [34]. However, patients with sleep breathing disorders may have more severe symptoms since arousal responses are depressed during sedation [34]. Therefore, it is reasonable to assume that 3 controls, who presented either low AHI (≤ 5) or no OSA/hypopnea during sedated sleep (Table 2), might not have OSA or hypopnea during natural sleep as well.

Fat composition and tissue stiffness

The prevalence of OSA is substantially increased over the past 2-3 decades due to the obesity epidemic, and more than 60% of OSA patients are obese [35]. There is a complex relationship between obesity and OSA. Obesity may accumulate adipose tissue around the pharyngeal structures, resulting in a narrow airway, increased critical pressure, reduced resting lung volume, and ultimately hypoxemia and alteration in metabolic hormones [36]. OSA may also accelerate weight gain, as sleep fragmentation often seen in OSA is associated with decreased leptin and increased ghrelin levels [37]. Disturbances in energy metabolism and insulin resistance have also been seen in OSA patients [38]. Furthermore, adipose tissue dysfunction resulting from OSA shows a striking resemblance to adipose dysfunction induced by obesity [39]. Therefore, OSA and obesity share similar pathophysiological mechanisms and may interact with and potentiate each other. Studying the direct interactions between OSA and obesity in humans could be difficult, as the interventions to alter OSA and/or obesity status are demanding, complex, and invasive. The results from MRI and USE in the present study shed some light on how fat composition and tissue properties of the tongue base and soft palate relate to OSA, leading to a better understanding of the pathophysiology of OSA. However, since no airflow data was simultaneously collected with these images, the associations between these imaging results and degrees of airway collapse and/or severity of OSA cannot be addressed.

The results suggest that percentage of fat composition increases from the middle region of the tongue base toward the most caudal region in all three groups. This result is consistent with an autopsy study in human cadavers [40]. The results also showed less fat deposition in the soft palate than the tongue base. However, the results were unable to demonstrate that obeseOSA minipigs contained significantly more fat tissue in the tongue base, soft palate,

and oropharyngeal wall than the controls as revealed in other studies in obese rats and humans [16,41]. In contrast, obeseOSA Panepinto minipigs even presented less fat composition as compared with controls and obeseOSA Yucatan minipigs, particularly in the caudal region of the tongue base and soft palate (Figure 10). Three reasons may be considered: First, there is a great age difference between Yucatan and Panepinto OSA minipigs (8-11 months vs. 78 months). Although fat tissue in the tongue increases with age in humans [42], this may not be the case in minipigs. In addition, the fat composition greatly varies in different breeds of pigs [43]. Second, since the fat composition was calculated as a percentage, rather than the actual amount, the sizes could have confounded the measurements as obesity usually leads to a large-sized tongue [44]. Third, no same-aged controls of Panepinto minipigs were included in the present study due to the unavailability. This limitation might lead to uncertainty about why the aged obeseOSA Panepinto minipigs presented lower fat compositions in these oropharyngeal structures.

The USE results revealed that overall, the tissue stiffness of the tongue was higher in the obeseOSA than control minipigs, significantly in the rostral region in obeseOSA Yucatan minipigs (Figure 11). These results are contradicted with the findings of more fat composition in obese rats and humans [16,41], as more fat infiltration in the tongue should lead to less tissue stiffness in the tongue [45]. However, the present findings are in agreement with a USE study in awake OSA patients, which found that the mid-sagittal tongue of OSA patients had significantly higher tissue stiffness than those in controls during normal breathing, and the highest value was in the tongue base during Müller's maneuver [46]. The tongue comprises the major dilator muscle of the oropharyngeal airway, i.e., genioglossus, and the tongue dilator muscle is synergically active with multiple oropharyngeal muscles such as palatal, hyoid, and pharyngeal muscles. While the components of the muscle and connective tissue contribute to the tissue stiffness, the higher muscle tone or isometric contraction caused by neural drive may also contribute to the tissue stiffness. Some electromyographic studies have also demonstrated that the motor units presented longer duration and larger size index in OSA patients [47]. Although MRI results show a trend that the fat composition gradually increases from rostral to caudal tongue base, no corresponding decreased trend of tissue stiffness was found in the USE analyses. In contrast, the rostral tongue of obese Yucatan minipigs presented the highest stiffness value. Therefore, the tissue components may not be the major determinant factor for the tissue stiffness measures in these minipigs. Since above-cited human OSA studies presented either tongue fat deposition or tissue stiffness alone, the direct correlation between these features is unknown. Therefore, combining both approaches in human subjects is the future direction to better understand the relationship of the tissue composition and stiffness in the tongue.

Respiratory internal kinematics and dynamic movements

The respiratory internal kinematics of the tongue base

and soft palate in obeseOSA minipigs are particularly relevant to the human OSA condition. A most recent clinical survey indicated that the prevalence of snoring in obese individuals is almost 100%, and 58% of them present severe degree of OSA with airway obstructions. These obstructions most often occur at the retro-palatal and retro-glossal levels [48]. A recent meta-analysis of 2,950 patients from 19 studies also showed the soft palate and tongue base were the two most common sites of airway obstruction. This meta-analysis also showed that the degree of tongue base obstruction was associated with the severity of OSA [49]. In the present study, both heavy snoring and severe OSA were identified in obese minipigs, but snoring did not occur in controls.

Nevertheless, the enhanced internal kinematics (Figure 8) and altered spatial relationship in the tongue base and soft palate during respiration is related to the presence of obesity/OSA, and this enhancement may have a compensatory effect on the potential oropharyngeal airway restriction or collapse.

It must be indicated that not all crystal recordings were successful due to the vulnerability of the implanted ultrasound crystals. Despite these, the results clearly reveal the respiratory characteristics of the internal kinematics in the tongue base and soft palate in controls and the differences with obeseOSA minipigs. As described in the methods, the recordings were performed under sedated sleep, instead of natural respiration in consciousness or sleep. Fortunately, the confounding effect of sedation and anesthesia on respiration has been proven to be minor [32,33,50], and the physical parameters between sedated and natural sleep present clear similarity in these normal and obese minipigs [18]. Therefore, this limitation could be considered minor but still needs to be confirmed during natural respiration when the technique becomes available.

All obeseOSA minipigs have larger respiratory movement of the soft palate, and audible heavy snoring accompanies significantly larger movements as seen in obeseOSA Panepinto minipigs, especially in the dorsal-ventral direction. Also, the movements are larger in expiratory than inspiratory phases. However, the movement ranges of the tongue base are much smaller and show no differences among the three groups and

between respiratory phases. It has been documented that the soft palate rises to touch the posterior pharyngeal wall, thus closing the nasopharynx to regulate airflow through nose and/or mouth [51]. This may suggest that the soft palate may be much more deeply involved in the genesis of snoring and OSA than the tongue base. This may also explain why uvulopalatopharyngoplasty (UPPP) which shortens and stiffens the soft palate by partially removing the uvula and reducing the edge of the soft palate, was a popular surgical intervention to treat OSA in the past. However, due to the suspendable, movable, and flexible nature of the soft palate, the focus of surgical intervention has shifted to the tongue base in recent years [52,53].

The present study demonstrated that the increase and decrease of each measured dimensional change in the tongue base do not compensate for each other to maintain the volume constant in the region circumscribed by the ultrasonic crystals as reported for the tongue body [25,54]. More importantly, the present study revealed that the elongation and widening of both the tongue base and soft palate may be the key players in leading or guiding airflow into the oropharyngeal airway. While both the dorsal tongue base and the soft palate widened, the ventral tongue base narrowed instead, along with anterior thinning and posterior thickening. Therefore, during inspiration, the cubic shape circumscribed by 8 implanted ultrasound crystals becomes an irregular trapezoid-like shape, featuring a longer and wider top but narrowed bottom, and further tapering sagittally from anteriorly decreased to posteriorly increased thicknesses as illustrated in Figure 15. At the same time, the soft palate extends in both length and width. These reciprocal dimensional changes in the shapes of the tongue base and soft palate expand the lumen of nasal/velar and oral pharynx thus increase the volume of the oropharyngeal airway for its patency, as seen in the airflow dynamics [21]. Thus, it is reasonable to speculate that an enlarged tongue base and/or soft palate due to obesity or other pathological conditions are predisposing factors of airway obstruction, particularly at the status of the decreased tone of the tongue and soft palate muscles during sleep [16].

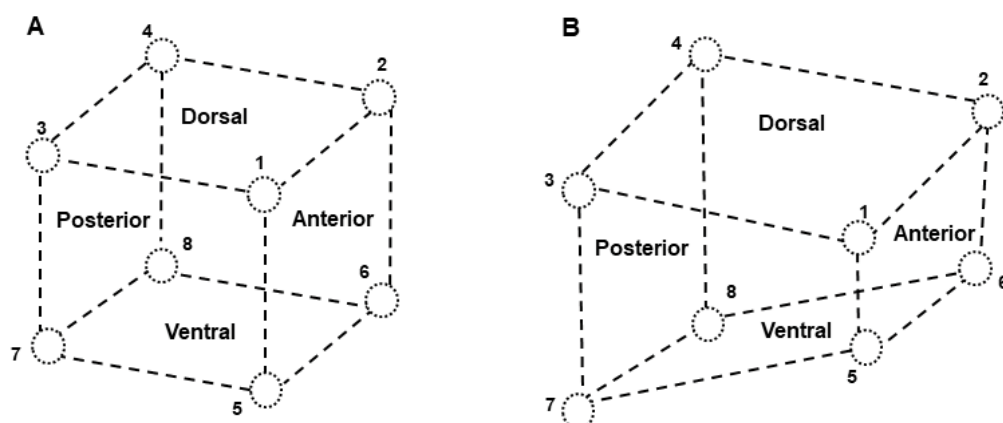


Figure 15: The illustration of the shape changes in the region of the tongue base circumscribed by the 8 ultrasonic crystals. (A) Initial shape after the crystal implantation; (B) The shape during inspiratory phase.

Respiratory airflow dynamics

Airway patency is controlled by several muscle groups that vary in activity with sleep state, within each breath, and phase of respiration [55]. Therefore, exploring the interaction between airway anatomy and its airflow dynamics is critical to understand respiratory physiology and pathophysiology. Airway anatomy and its neuromuscular control have been implicated in the pathophysiology of OSA. Studies have shown that the structural change of narrowing the upper airway could additionally predispose the airway to collapse [56]. Although the partial or complete collapse of pharyngeal airway is widely accepted as a primary cause of OSA, the etiology of the airway collapse is poorly understood and airflow dynamics leading to airflow reduction or blockage is hardly predicted. Therefore, image based CFD is an ideal modeling tool that can generate a wealth of data about the airway flow field and provide important insight regarding the pathophysiology of OSA. CFD has been used to characterize the airflow in various airway models reconstructed from MRI or CT images, which mostly used laminar or steady Reynolds-Averaged Navier-stroke (RANS) model as used in the present study. Given the great variations in the shape and size of airway and changes in relation to various physiological or pathological conditions, these numerical algorithms need to be simulated and validated by actual respiratory airflow parameters to accurately predict the airflow dynamics from animal experiments or clinic setting [57-60]. This emergent approach of imagebased CFD has been applied for preoperative prediction of airway changes after maxillomandibular advancement surgery [59] and assessment of upper airway response to oral appliance treatment for OSA [60]. However, in most of these image-based CFD studies, the simulation of the model has been established by the respiratory airflow parameters measured from an identical or similar physical model rather than the modeled subject. In the present study, the CFD models were established using meshing 3D segmented data from MRI images. These models were simulated and validated using the inspiratory and expiratory airflow velocity from the same representative of obeseOSA and control minipigs as those for the model establishment. Thus, the present study provides validated CFD models to depict airflow dynamics in the nasal/velar and oral pharyngeal airways in normal and obeseOSA minipigs.

The present study shows that obeseOSA minipigs presented significantly small tidal volume and slower airflow velocity of both inspiration and expiration phases compared to controls (Figure 13). The CFD model identified a ~25% increase of airflow velocity at the center of the narrowest portion of oropharyngeal airway located at the transitional region of nasal/velar and oral pharynx. However, the overall volumetric flow rate has decreased by 30% (Figure 13). This contradiction should be attributed to the methods of the actual airflow velocity measurement using Peumotach system and FEM simulation. In the former, the velocity was measured by the sensor located at the end of mask, but in the latter, the velocity was measured at each meshed location of pharyngeal airway.

The CFD model of the present study identified that the narrowest portion of pharyngeal airway in obeseOSA minipig

is located at the transitional region of the nasal/velar and oral pharynx, i.e. the caudal end of soft palate (retropalatal) and beginning of oral pharynx (retroglossal). This finding is consistent with a number of clinical studies [61]. Therefore, various surgical treatments have been focused on reducing the tissue mass of this region for airway expansion [62] and the study also found that this region was mostly affected by the type of masks [63].

Turbulence would have effectively contributed to an added resistance to the overall airflow, thereby reducing the air volume exchange during respiration in obeseOSA minipigs. However, the observed airflow patterns were laminar in nature for both control and obeseOSA minipigs. This observation may lead to the hypothesis that turbulence is expected in the obeseOSA minipigs due to abnormal variations in the pharyngeal airway. The reasons for the lack of turbulence observed are two-fold. The formation of turbulence requires a combination of two parameters: An abrupt dimensional change in the airflow pathway and sufficiently high airflow velocity. In the case of the obeseOSA minipig, a narrower gap was seen in the pharyngeal airway, resulting in a larger velocity gradient across the gap of 25% (vertical arrow in Figure 14). However, this narrowing and the subsequent expansion along the airflow path are not abrupt enough for the given inspiratory and expiratory airflow velocities to create turbulence. Despite the increased velocity in this local region, the airflow tidal volume and overall airflow velocity were significantly lower in obeseOSA minipigs compared to control minipigs (Figure 13).

With the lack of turbulence observed in obese/OSA minipigs, the lower air volume exchange during respiration and airway collapse during apnea/hypopnea episodes is likely the result of a lack of "air pumping efficiency" to compensate for the narrower air pathway. Since a smaller cross section restricts the volumetric airflow, the narrower airflow pathway reduces the total volumetric airflow. Although there is some compensation in the increased airflow velocity locally through the narrowest region of the nasopharynx, the volumetric airflow rate remains reduced. In obeseOSA minipigs with a narrower airflow pathway, the volumetric airflow rate would naturally be lower than controls unless their lungs pumped more air to compensate for the reduced airflow rate. The fact that the measured tidal volumes and inspiratory airflow velocity were significantly lower in obeseOSA minipigs indicated that its lungs were not pumping fast enough for its airflow volume to match that of the controls.

Hence, the combination of measured airflow parameters and airflow characteristics simulated by CFD provided the following insights. The narrowing of the airflow pathway in obeseOSA minipig does not contribute to any turbulent airflow, which would have resulted in added airflow resistance during respiration and contributed to the airway collapse. Rather, the drop in the air volume exchange in obese/OSA minipigs relative to the non-obese controls is due to the added resistance by the narrower air pathway. In conjunction with the narrower air pathway in obese/OSA minipigs, their lungs are not strong enough to overcome this resistance so as to match the airflow rate of non-obese controls.

Although the CFD model assumes that the airway tissues are stationary during each phase of respiration and does not model the dynamic airway collapse, the overall airflow rate of obeseOSA minipigs results in a significant decrease in the air volume exchange. By identifying the presence of an increase in airflow speed and isolating its location, this CFD simulation provides the framework for succeeding OSA research, in particular for preventing the airway collapse in OSA.

Conclusions

The present study concluded that 1) The obese minipig presents naturally occurring OSA, thus is an ideal large animal model for obese-related OSA study; 2) The enhanced respiratory internal kinematics in the tongue base and soft palate are related to the presence of obesity/OSA, and these enhancements may have compensatory effects on the potential oropharyngeal airway restriction or collapse; 3) The fat composition is highest in the posterior 1/3 of the tongue base, regardless of the presence of obesity and OSA; 4) ObeseOSA minipigs have stiffer tongue tissue than the controls, particularly in the rostral region of the tongue, which may result from altered neuromuscular drive; 5) The moving range of the soft palate is significantly more extensive in obeseOSA than control minipigs. This increased range may also have a compensatory effect on the potential oropharyngeal airway restriction or collapse; 6) The reduced dimension of the nasal/velar pharyngeal airway and respiratory tidal volumes, and increased airflow velocity present in obeseOSA minipigs; 7) The local higher airflow velocity in the narrowest part of the oropharyngeal airflow pathway exists in obeseOSA minipigs but no local turbulence is produced.

Author Contributions

ZJL conceived the study, interpreted data, and wrote the manuscript; MYA and MZD collected data and performed data analysis; WEM interpreted data and revised the manuscript. All authors contributed to the final manuscript and have approved the final version for this submission.

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Conflict of Interest Statement

The authors declare no conflicts of interest with the

content of this article. The authors have no competing interests or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

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