TONGUE FAT AND ITS RELATIONSHIP TO OBSTRUCTIVE SLEEP APNEA

Tongue Fat and its Relationship to Obstructive Sleep Apnea

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Study Objectives: The objective of this study was to determine whether tongue fat is increased in obese sleep apneics compared to obese subjects without sleep apnea. We hypothesized that excess fat is deposited in the tongue in obese patients with sleep apnea.

Design: Case-control design.

Setting: Academic medical center.

Patients: We examined tongue fat in 31 obese controls (apnea-hypopnea index, 4.1 ± 2.7 events/h) and 90 obese apneics (apnea-hypopnea index, 43.2 ± 27.3 events/h). Analyses were repeated in a subsample of 18 gender-, race-, age-, and BMI-matched case-control pairs.

Interventions: All subjects underwent a MRI with three-point Dixon magnetic resonance imaging. We used sophisticated volumetric reconstruction algorithms to study the size and distribution of upper airway fat deposits in the tongue and masseter muscles within apneics and obese controls.

Measurements and Results: The data supported our *a priori* hypotheses that after adjustment for age, BMI, gender, and race, the tongue in apneics was significantly larger (P = 0.001) and had an increased amount of fat (P = 0.002) compared to controls. Similar results were seen in our matched sample. Our data also demonstrate that within the apneic and normal tongue, there are regional differences in fat distribution, with larger fat deposits at the base of the tongue.

Conclusions*:* There is increased tongue volume and deposition of fat at the base of tongue in apneics compared to controls. Increased tongue fat may begin to explain the relationship between obesity and obstructive sleep apnea.

Keywords: obstructive sleep apnea, tongue fat

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INTRODUCTION

Obstructive sleep apnea (OSA) is a major public health burden affecting greater than 15 million adult Americans—more worldwide—and is associated with important medical consequences.[1-5] The prevalence of OSA is increasing, mirroring the rising weight of the average individual, as obesity is the strongest risk factor for the development of OSA.[4,6,7]

The mechanisms by which obesity confers risk for OSA, however, are essentially unknown. We hypothesize that fat deposition will increase overall tissue volume in upper airway structures, thereby playing an important role in the pathogenesis of OSA. Based on previous studies,[8,9] we hypothesized that fat deposition in the tongue could be a major link between obesity and OSA. An autopsy study demonstrated that the human tongue has a high percentage of fat (with more fat localized at the tongue base), and that tongue weight and tongue fat percentage were positively correlated to the degree of obesity.[10] However, this study did not specifically examine patients with OSA. A mutant mouse with obesity, the New Zealand Obese (NZO), has also been shown to have increased fat deposition in the tongue compared to wild-type controls.[8,11] Tongue fat in the NZO mice was associated with a narrowed upper airway.[9]

The primary goal of this study was to identify alterations in fat deposition within the tongue of obese apneics in comparison to obese subjects without sleep apnea, using the three-point Dixon method (a method for fat/water discrimination).[12] The Dixon method has been validated in fat/water phantoms, and this imaging modality has been shown to be highly reproducible and accurate for determining fat volumes.[9] We used a case-control design to examine our *a priori* hypotheses that: (1) the volume of fat within the tongue is increased in obese patients with OSA in comparison to obese subjects without OSA, suggesting that increased tongue fat volume is an independent OSA risk factor; (2) the percentage of fat in the tongue is greater than in other upper airway muscles (e.g., masseters); and (3) based on the previously mentioned autopsy study,[10] tongue fat has a specific regional topography, such that it is more prominent at the base of tongue. Portions of this investigation have been previously presented as an abstract.[13]

METHODS

Subjects

The present study used a case-control design in overweight or obese apneics and non-apneics. The University of Pennsylvania (Philadelphia, PA, USA) Institutional Review Board for human studies approved the protocol (protocol numbers 808496 and 809398), and written informed consent was obtained from each subject. Overweight or obese cases were recruited primarily from the Center for Sleep and Circadian Neurobiology (Philadelphia, PA) outpatient practice. Cases

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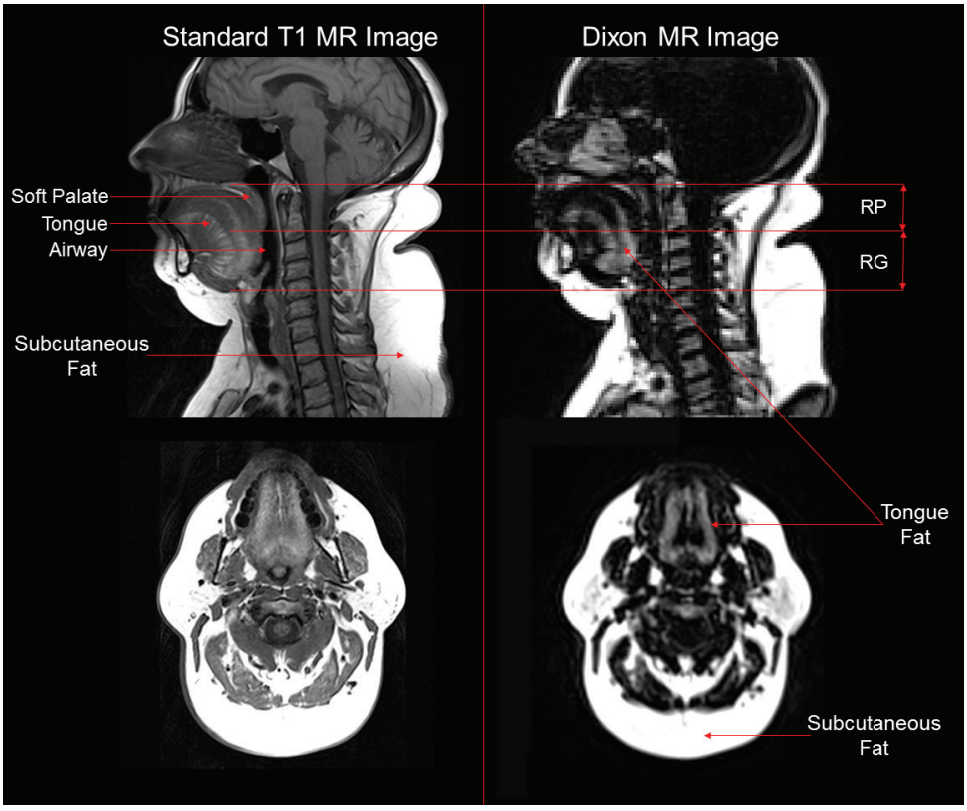

Figure 1—Comparison of tongue fat using standard T1-weighted spin echo MR imaging vs. fat-weighted Dixon MR imaging sequences in an apneic patient. Anatomic definitions of the upper airway regions are demonstrated: retropalatal (RP)—from level of hard palate to caudal margin of soft palate; and retroglossal (RG)—from caudal margin of soft palate to base of tongue. Soft palate, tongue, tongue fat, and airway are denoted with arrows. Both sagittal images are mid-sagittal and axial images are shown at same level of (mid) tongue. Note that fat deposits in tongue are more easily visualized using Dixon MR imaging sequence than the spin echo sequence.

had an apnea-hypopnea index (AHI) ≥ 15 events/hour. Overweight or obese control subjects (BMI ≥ 28.7 kg/m^2, AHI ≤ 10 events/h) were recruited in the Philadelphia area. Subjects with 10 < AHI < 15 were considered indeterminate and not included in this study. In addition to the overall case-control sample, we created a matched subsample of gender, race, age (within 10 years), and BMI (within 2.5 kg/m^2) case-control pairs for complementary analyses.

Polysomnography

Standard polysomnograms were performed as described in our previous studies.[14] See supplemental material for details on conduct of sleep studies and definition of events and scoring.

Upper Airway MRI Acquisition

Using a 1.5 Tesla MAGNETOM Espree Scanner (Siemens Medical Systems, Malvern, PA), high resolution upper airway MR imaging was performed identically in all subjects. See supplemental material for details regarding MR imaging sequences utilized.

Anatomic Definitions, Measurements, and Analysis

Using Amira 4.1.2 (Visage Imaging, San Diego, CA), MR images of the upper airway were manually examined at the Pulmonary Sleep Imaging Center (University of Pennsylvania). The MR imaging analysis was split into 2 domains: volumetric analysis of soft tissues (tongue and masseter) and intramuscular fat (tongue and masseter) quantification. Soft tissue volumes were obtained from the standard T1 spin-echo axial images. The segmented boundaries of the tongue and masseter were determined from the spin-echo images and then superimposed on the Dixon images for proper tissue visualization. Fat-weighted Dixon images provide a clear indication of adipose tissue, but the boundaries of soft tissues are not as distinct in comparison to the standard spin-echo images (Figure 1). Intramuscular fat was then determined using a thresholding method to differentiate fat from the rest of the tongue. The correct grayscale setting to segment tongue fat was chosen based on identifying the appropriate grayscale intensity of the surrounding subcutaneous and neck fat (Figure S1, supplemental material). The topographic distribution of tongue fat was further determined by subdividing the tongue into 8 sections—4 within the retropalatal and 4 within the retroglossal regions of the tongue—these regions were based on the craniocaudal axis from the mid-sagittal MR image (Figures 1 and 2). The retropalatal region of the tongue was defined from the most rostral portion of the hard palate to the most caudal tip of the soft palate. The retroglossal region of the tongue was defined from the most caudal margin of the soft palate to the base of the epiglottis (Figure 1)

Overall fat within the masseter muscle, within the axial range of the tongue, was examined as a comparable upper airway muscle using the same method and grayscale settings. We analyzed 20 subjects and showed there were no differences in the volume of the masseter, volume of fat in the masseter or masseter fat percentage between the right and left masseter muscles. Therefore, fat percentage was determined from subjects' left masseter muscles. All MR imaging analyses were performed by one trained technologist blinded to the subject's status (apneic or control) and supervised by one investigator (RJS).

Reproducibility of the Dixon MRI was assessed using intraclass correlation coefficients for MR structures (soft tissue volumes, tongue fat, and airway volumes) based on measurements performed at 2 different time points in 10 different subjects. Accuracy of our fat volume estimates were assessed by performing Dixon MR imaging of a hamburger (Figure S2, supplemental material) and steak before and after injection of a known volume of fat (lard) into the tissue (3 cc added to the hamburger and 6 cc added to the steak) and comparing the

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resulting estimates to the known quantity. We also placed a fat and water phantom next to each tissue.

Statistical Analysis

Analyses were performed using Stata, Version 12 (StataCorp, 2011, Stata Statistical Software: Release 12. College Station, TX) or SAS Software, Version 9.3 (SAS Institute Inc., Cary, NC). Chi-square tests and unpaired t-tests examined differences in demographics, soft tissue, and intramuscular fat volumes between OSA and control subjects. Differences between OSA and controls adjusted for age, BMI, gender, and race were assessed using an analysis of covariance (ANCOVA). Adjusted linear associations of tongue fat on AHI were examined using partial Pearson correlations. The tongue was divided into 4 subsections per region in order to conduct within tongue region analyses. Regional differences in tongue fat within apneics and controls were assessed using repeated measures ANOVA, accounting for the multiple regions per subject. To assess analysis reproducibility, intraclass correlation coefficients were computed. This study was adequately powered (80%) to detect medium effect sizes (Cohen's d = 0.6) or greater, with actual power achieved for the primary outcome of volume of tongue fat being 89%.

To further control for the impact of imbalance between cases and controls in the primary covariates of age, BMI, gender, and race, we performed a secondary analysis using a matched case-control subset. Matching was conducted based on an exact match of gender and race, age within 10 years, and BMI within 2.5 kg/m^2, and resulted in 18 matched case-control pairs for this secondary analysis. Differences in demographic characteristics between matched cases and controls were assessed using paired *t*-tests for continuous variables and McNemar test for categorical variables. Unadjusted analyses examining the differences in soft tissue and intramuscular fat volumes between matched OSA cases and controls were performed using paired *t*-tests. Analyses adjusted for the small remaining differences in BMI and age after matching were performed using a linear regression, with the dependent variable equal to the difference in soft tissue or intramuscular fat for each case-control pair (i) as follows:

$$E(\textit{Phenotype Difference}_i) = \beta_0 + \beta_1(\textit{BMI Difference}_i) + \beta_2(\textit{Age Difference}_i)$$

We then used a Wald test to determine whether the intercept (β_0), which represents the expected phenotype difference, was equal to 0. We note that in the absence of adjustments, this model is equivalent to the paired *t*-test.

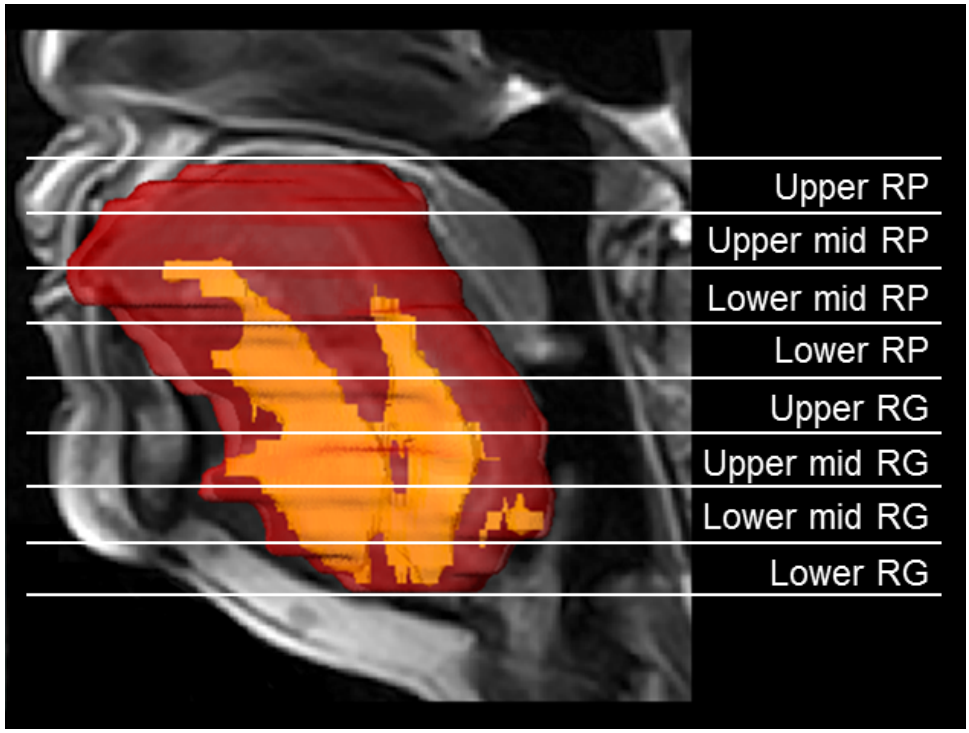

Figure 2—Representative three-dimensional volumetric reconstruction of tongue (red) and fat within tongue (yellow) from series of 3-mm contiguous axial MR images superimposed on a midsagittal image; 8 sections of apneic tongue (red) including tongue fat (yellow), 4 within RP (retropalatal) region and 4 within RG (retroglossal) region of tongue. There is substantially more fat at base of tongue.

RESULTS

Demographics of Cases and Controls

Descriptive statistics of our overall case-control sample and our matched subset are shown in Table 1. Our case-control study was comprised of 90 patients with OSA and 31 subjects without OSA. Patients with OSA were required to have an AHI ≥ 15 (mean ± SD: AHI of 43.2 ± 27.3 events/h) and control subjects were required to have an AHI ≤ 10 (AHI of 4.1 ± 2.7 events/h) (Table 1). We were able to recruit both overweight and obese apneics (mean ± SD BMI of 39.1 ± 8.3 kg/m^2) and controls (BMI of 34.1 ± 4.8 kg/m^2). Cases were slightly older (P = 0.004) and also heavier (P < 0.001) than control subjects, although our control subjects were still quite obese. Both groups had BMI ranges that substantially overlapped, allowing us to statistically adjust for differences in BMI. There were no significant differences between apneics and controls in the subject ratios of gender (P = 0.162) or race (P = 0.156).

To further mitigate the impact of the covariate differences between cases and controls on our results, we also performed a secondary analysis on a subset of 18 BMI (± 2.5 kg/m^2), age (± 10 years), gender, and race-matched case-control pairs. As expected, the 2 groups no longer differed in terms of BMI (P = 0.808) or age (P = 0.827) after matching (see Table 1).

Volumetric MR analysis of Tongue and Tongue Fat Volumes

The primary focus of this study was to identify alterations in tongue fat using Dixon MR imaging. In order to make sure these data were reproducible, intraclass correlation coefficients

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Table 1—Demographics of case and control subjects.

	Overall Case-Control Sample					Matched Case-Control Sample				
	Apneics (n = 90)		Controls (n = 31)			Apneics (n = 18)		Controls (n = 18)		
Factor	Mean	SD	Mean	SD	P†	Mean	SD	Mean	SD	P‡
Age, years	49.6	9.9	41.6	13.2	**0.004**	50.3	2.8	50.1	4.4	0.827
BMI, kg/m^2	39.1	8.3	34.1	4.8	**< 0.001**	33.6	4.5	33.5	4.1	0.808
AHI, events/h	43.2	27.3	4.1	2.7	**< 0.001**	28.2	12.8	3.9	2.5	**< 0.0001**
Gender, M:F	42:48		10:21		0.162	5:13		5:13		1.000
Race, C:AA	39:51		18:13		0.156	9:9		9:9		1.000

AHI, apnea-hypopnea index; BMI, body mass index; C, Caucasian; AA, African American. Significant differences shown in bold. † P-value from *t*-test (for continuous variables) or χ^2 test (for categorical variables); ‡ P-value from paired *t*-test or McNemar test.

Table 2—Comparison of muscle volumes and tongue fat in case and control subjects.

	Apneics (n = 90)		Controls (n = 31)			
Soft Tissue Volume	Mean	SD	Mean	SD	P†	P‡
Tongue, mm^3	101,193	17,651	85,542	13,813	**< 0.001**	**0.001**
Tongue fat, mm^3	32,791	9,175	23,390	5,511	**< 0.001**	**0.002**
Tongue fat, %	32.6	7.9	27.7	6.7	**0.002**	0.089
Tongue lean mass, mm^3	68,401	15,336	62,152	13,370	**0.046**	0.123
Left masseter, mm^3	16,204	6,633	14,517	6,342	0.214	0.794
Left masseter fat, mm^3	786	859	599	766	0.262	0.118
Left masseter fat, %	5.2	5.9	4.8	6.1	0.794	0.384

Significant differences are presented in bold. † P-value from *t*-test; ‡ P-value after adjustment for age, BMI, gender, and race.

from repeated measures within 10 subjects were calculated for MR structures; all coefficients were > 0.95, indicating a highly reproducible analysis. To measure accuracy, we used Dixon MR imaging to quantify fat volumes in a hamburger (Figure S2) and steak before and after an injection of a known volume of fat (3 cc lard added to the hamburger and 6 cc added to the steak). Our data show that we can accurately quantify the amount of fat added to these tissues using Dixon MR imaging (3.03 cc and 5.96 cc, respectively; see also Table S1, supplemental material).

Obesity Measures and Tongue Fat

We assessed the relationship between clinical and MRI measures of obesity and tongue volume, tongue fat volume, and tongue fat percentage (Table S2, supplemental material). In general, patients with more obesity had larger tongue and tongue fat volumes, as well as higher percentages of tongue fat. There were significant correlations (0.44; P < 0.0001) between visceral fat in the abdomen and tongue fat.

Differences in Tongue Fat Volume between Apneics and Controls

Apneic subjects had more tongue fat than controls (see Table 2 and Figure 3). An apneic with an enlarged tongue and increased tongue fat deposition is shown in comparison to an obese control in Figure 4. In all subjects, using quantitative volumetric measurements, apneics were shown to have significantly greater tongue volumes (P < 0.001), greater tongue fat (P < 0.001), and a greater percentage of tongue fat (P = 0.002) than controls (see Table 2 and Figure 3). After adjustment for age, BMI, gender, and race, tongue volume, and tongue fat remained significantly increased in apneics compared to controls (P = 0.001, P = 0.002, respectively); this was not true for tongue fat percentage (P = 0.089).

When repeating these analyses within our age-, BMI-, gender-, and race-matched case-control subsample, we observed similar differences between apneics and controls (Table 3). Apneics had significantly larger tongue volumes (P = 0.022) and greater tongue fat (P = 0.010) than controls (see Table 3). While the difference in tongue fat percentage was not significant in our reduced sample of 18 matched pairs, the magnitude of the difference was similar in the matched subset and the overall sample (4.3% vs. 4.9%).

We also examined tongue lean mass (Tables 2 and 3). In the entire group, in the unadjusted model there was a borderline significant (P = 0.046) increased tongue lean mass in apneics compared to controls (Table 2). However, this difference was lost after controlling for age, BMI, gender, and race. There were no statistically significant differences in tongue lean mass in the matched subset (Table 3). These data support our hypothesis that tongue fat is the major factor in explaining the increase tongue volume in apneics compared to controls, although differences in tongue lean mass could play a lesser role.

Distribution of Fat within the Tongue

For the topography of tongue fat, we examined the data both within groups (apneic or control) and between groups (apneic

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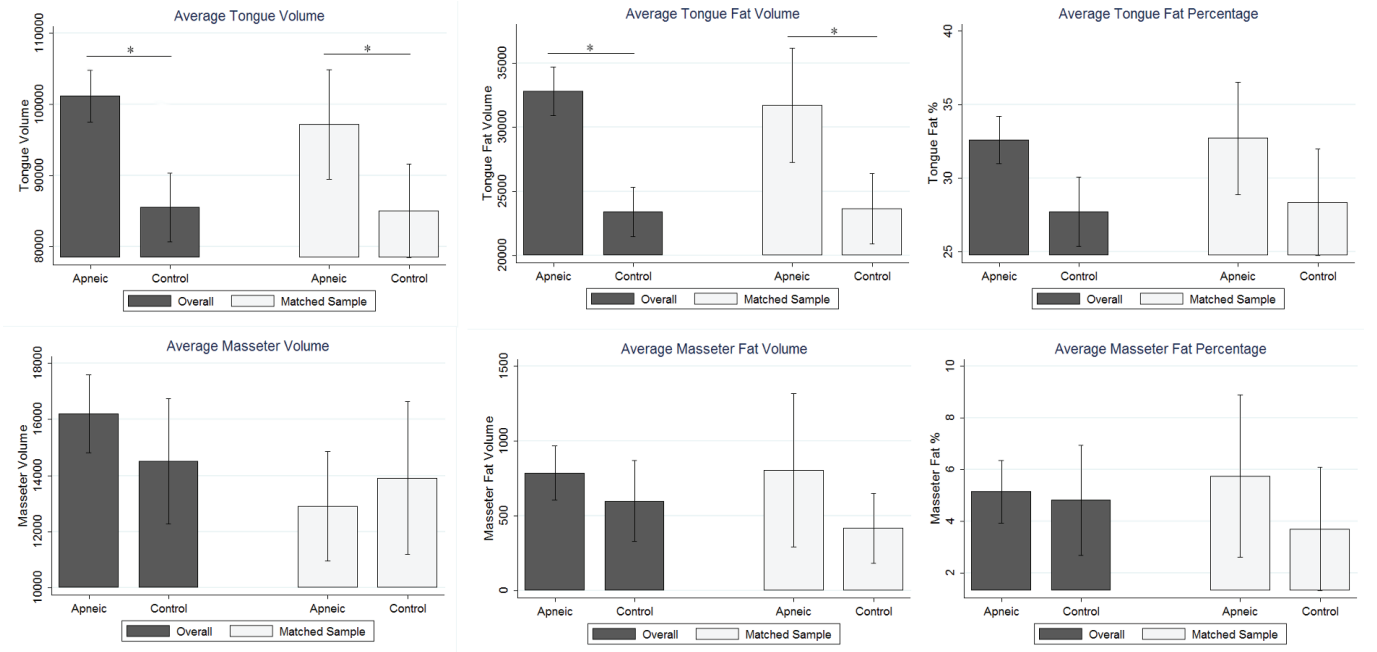

Figure 3—The mean and 95% confidence intervals for our primary outcomes of interest are shown for cases and controls, both in our overall sample and for the age, BMI, gender, and race-matched pairs. *After adjustment for age, BMI, gender, and race, we observe significant differences between cases and controls for tongue volume and tongue fat, both in the overall population (P = 0.001 and P = 0.002, respectively) and in our matched sample (P = 0.022 and P = 0.010, respectively). There were no significant differences in masseter fat between the apneics and controls. In all subjects tongue fat % is greater than masseter fat %.

vs. control). There were significant differences in intramuscular fat percentage among the 4 sections ($P < 0.001$) of the RP and RG regions of the tongue, as well as across all 8 sections ($P < 0.001$) within apneics and within controls, indicating heterogeneous fat distribution in both groups (see Figure 5 and Table 4).

Examination of differences between groups showed that the percentage of fat in the retroglossal region of the tongue was significantly greater in apneics than in controls ($P = 0.023$), whereas there were no significant differences in the percentage of tongue fat in the retropalatal region ($P = 0.993$) after adjustment for age, BMI, gender, and race (Table 4). When examining the differences in more detail, the lower mid and lower sections of the retroglossal region of the tongue had significantly higher percentages of fat in apneics than controls after adjustments for age, BMI, gender, and race ($P = 0.003$ and $P < 0.001$, respectively) (Table 4). These data indicate that there are region

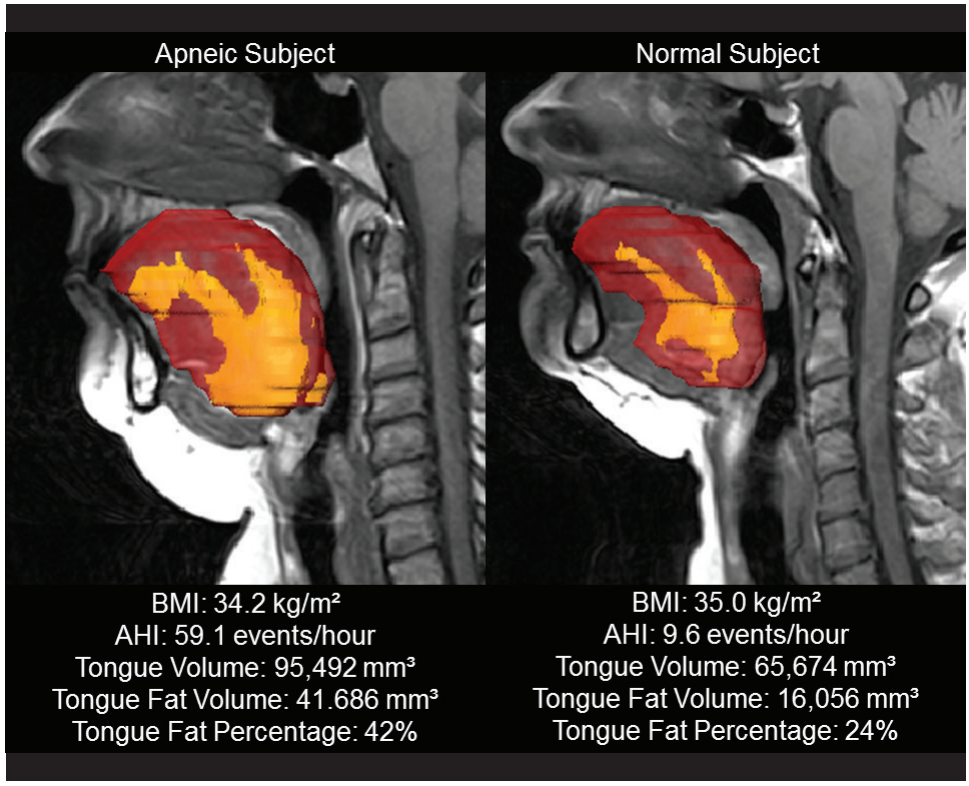

Figure 4—Representative three-dimensional volumetric reconstructions of tongue (red) and fat within tongue (yellow) from series of 3-mm contiguous axial MR images superimposed on midsagittal images in BMI-matched post-menopausal female patient with OSA (left) and post-menopausal female control subject (right) (subjects also matched for age and ethnicity). The apneic tongue is much larger and there is increased tongue fat deposition throughout the apneic tongue.

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Table 3—Comparison of muscle volumes and tongue fat in BMI-, age-, gender-, and race-matched case-control pairs.

	Apneics (n = 18)		Controls (n = 18)			
Soft Tissue Volume	**Mean**	**SD**	**Mean**	**SD**	**P†**	**P‡**
Tongue, mm^3	97,188	16,626	85,067	14,199	**0.014**	**0.022**
Tongue fat, mm^3	31,712	9,695	23,638	5,979	**0.009**	**0.010**
Tongue fat, %	32.7	8.3	28.4	7.8	0.147	0.142
Tongue lean mass, mm^3	65,476	14,404	61,429	14,684	0.390	0.436
Left masseter, mm^3	12,906	4,230	13,910	5,903	0.528	0.450
Left masseter fat, mm^3	806	1,109	417	508	0.173	0.196
Left masseter fat, %	5.8	6.8	3.7	5.2	0.266	0.291

Significant differences are presented in bold. † P-value from paired *t*-test; ‡ P-value resulting from regression analysis on difference in outcome, after adjustment for age and BMI differences.

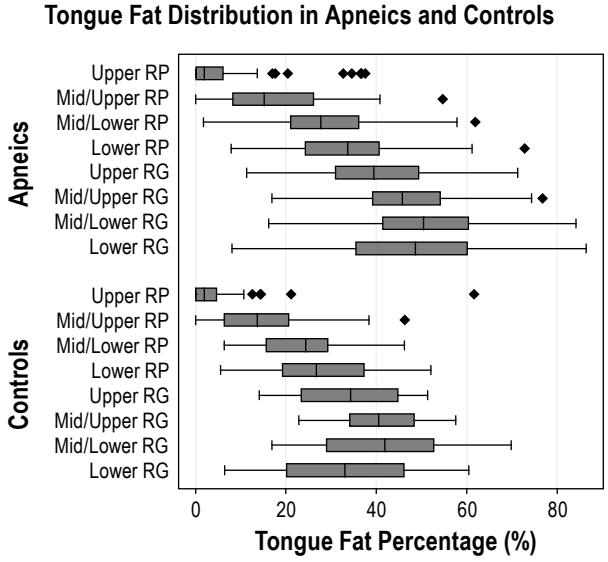

Figure 5—Graphical box and whisker plot comparison of tongue fat distribution within apneics and controls. The gray box represents the interquartile range (IQR = 75th Percentile – 25th Percentile), with the black line within the box showing the median value. The "whiskers" extend from the box either to the respective minimum or maximum value, or 1.5 × IQR from the 25th and 75th percentiles if points lie outside this range. After adjustment for age, gender, BMI, and race, the percentage of intramuscular fat in the lower mid RG (P = 0.003) and lower RG (P < 0.001) regions of tongue is significantly greater in apneics than in controls. RP = retropalatal and RG = retroglossal.

specific differences in the distribution of RP and RG tongue fat within apneics and controls as well as between apneics and controls.

Within our matched case-control sample (Table S3, supplemental material), we once again observed a significantly higher percentage of fat in apneics compared to controls within the lower RG region (P = 0.010), but not for the lower mid RG (P = 0.072). There was a significant difference in fat percentage within the lower mid RP region (P = 0.044). Therefore, our matched sample results support the overall observation of an increased deposition of tongue fat within the lower regions of the retroglossal region of the tongue in apneics.

Relationship between AHI and Tongue Fat

We next assessed whether there was correlation between continuous AHI and tongue volume, fat volume, and fat percentage. There was a significant positive correlation between AHI and both tongue volume (P = 0.0001) and tongue fat (P = 0.001) after adjustment for age, BMI, gender, and race. There was a nonsignificant correlation for tongue fat percentage (P = 0.117) (Table 5). There were no statistically significant correlations between AHI and any of the RP regional tongue fat percentages (Table S4, supplemental material). We observed significant correlations between AHI and both the upper (P = 0.029) and lower-mid (P = 0.034) RG regions. We also observed borderline nonsignificant correlations for the total RG fat percentage (P = 0.068) and the upper-mid RG region (P = 0.073) (see Table S4).

Differences in Fat Volumes in the Masseter Muscles

While we saw differences in fat in the tongue between apneics and controls, there were no differences in fat volume (P = 0.118) or fat percentage (P = 0.384) between apneics and controls in the masseter muscle (a control muscle) after adjustment for age, BMI, gender, and race (Figure 3 and Table 2). Similarly, there was no significant difference in the matched case-control subset for either the volume (P = 0.196) or percentage (P = 0.291) of fat in the masseter muscle (Figure 3 and Table 3). Masseter volume, masseter fat, and masseter fat percentage were not statistically significantly correlated to AHI (Table 5). The percentage of fat within the apneic tongue (32.6%) was significantly greater (P < 0.001) than in the masseter muscle (5.2%) (Table 2). This was also true in controls, where the percentage of fat in the tongue (27.7%) was significantly greater (P < 0.001) than in the masseter muscle (4.8%).

DISCUSSION

This is the first study showing that the amount of tongue fat in obese apneics is greater than in obese controls. We have demonstrated that (1) apneics have enlarged tongue volumes and increased fat within the tongue compared to control subjects, after adjustment for age, BMI, gender, and race; this was true in the entire sample and in a secondary analysis in a well-matched case-control subset; (2) there was more fat in the tongue than in a control upper airway muscle (masseter) in both apneics and

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Table 4—Comparison of tongue fat percentage and distribution within retropalatal (RP) and retroglossal (RG) regions in case and control subjects.

	Apneics (n = 90)		Controls (n = 31)			
Tongue Fat Percentage	**Mean**	**SD**	**Mean**	**SD**	**P†**	**P‡**
Total RP, %	24.4	9.0	21.0	8.5	0.065	0.993
Upper RP, %	5.3	8.7	5.6	11.7	0.887	0.137
Upper mid RP, %	17.4	11.7	15.2	10.8	0.334	0.739
Lower mid RP, %	28.6	11.3	23.4	9.4	**0.015**	0.454
Lower RP, %	33.3	12.8	27.9	11.4	**0.032**	0.714
Total RG, %	45.5	11.7	37.6	8.7	**< 0.001**	**0.023**
Upper RG, %	40.4	12.9	33.9	12.0	**0.013**	0.365
Upper mid RG, %	47.1	11.9	41.0	9.8	**0.007**	0.122
Lower mid RG, %	50.5	13.8	40.3	13.4	**0.001**	**0.003**
Lower RG, %	47.3	19.7	33.5	14.2	**< 0.001**	**< 0.001**

Significant differences are presented in bold. †P-value from *t*-test; ‡P-value after adjustment for age, BMI, gender, and race. RP, retropalatal; RG, retroglossal; SD, standard deviation.

controls; (3) tongue fat percentage in apneics was increased in specific locations of the tongue (greater in the retroglossal region); (4) tongue fat volume correlates with AHI and BMI. Our data provide evidence of a novel mechanistic pathway which may explain the relationship between obesity and sleep apnea.

Importance of Tongue Fat

Previous autopsy[10] and fast spin echo imaging[15] studies have shown that the tongue, a primary upper airway soft tissue risk factor for OSA, has a high percentage of fat. These studies, however, did not specifically examine subjects with OSA. We have shown that in obese apneics, the tongue has a very high percentage of intramuscular fat (32.6%). Our reported tongue fat percentages in controls (27.7%) were slightly greater, on average, than those reported in the previous autopsy[10] and imaging[15] studies, likely because our recruited population was heavier. In contrast, we found that the masseter muscle did not have a high percentage of fat, suggesting that fat is not deposited uniformly in muscles of the upper airway and that the tongue may be a unique reservoir for fat deposition. Our study also found that the apneic tongue is enlarged and composed of a larger amount of intramuscular fat than the tongue in controls. Although the differences in the overall tongue fat percentage between cases and controls were not statistically significant after covariate adjustment (32.6% vs. 27.7%, P = 0.089), we did observe statistically significant differences between apneics and controls in fat percentage within the RG region of the tongue, particularly near the base. Within these same apneics and controls, no differences were found in masseter muscle volume and masseter fat composition.

These findings raise important questions as to why intramuscular fat percentage differs so greatly between muscles of the upper airway and what factors are driving the preferential deposition of fat in the tongue. We believe increased fat in the apneic tongue may in part be explained by the role of genetics. Genetic heritability of fat distribution phenotypes has been demonstrated,[16] and deposition of total, trunk, and lower body fat has been shown to be under genetic control.[17] Fat deposits in the abdomen in men and neck in women have been previously shown to have obesity-related influences on OSA.[18] The heritability of fat deposits in the upper airway muscles (tongue) has yet to be studied. Although we have shown that tongue volume is heritable,[19] patients with OSA appear to preferentially deposit fat in the tongue as well as in the soft palate.[20] Increased intramuscular tongue fat and fat percentage, particularly at the base of tongue, may be important intermediate phenotypes for OSA.

Table 5—Relationship between AHI and fat measurements in the tongue and masseter in all subjects.

Primary Soft Tissue	Partial Rho	P†
Tongue, mm^3	**0.35**	**0.0001**
Tongue fat, mm^3	**0.30**	**0.001**
Tongue fat, %	0.15	0.117
Left masseter, mm^3	0.05	0.599
Left masseter fat, mm^3	0.05	0.632
Left masseter fat, %	0.04	0.644

Significant correlations are presented in bold. †P-value after adjustment for age, BMI, gender, and race.

Role of Tongue Fat in the Pathogenesis of OSA

The tongue is known to be the most important pharyngeal dilator muscle.[21] It is a unique freely moving muscle that, unlike other muscles, is anchored to bone at only one end, the base, by four extrinsic (external bony origin and insertion into the tongue base) muscles (genioglossus, hyoglossus, styloglossus, palatoglossus). These extrinsic muscles are responsible for tongue positioning,[22] while the four intrinsic (origin and insertion within the tongue) muscles (superior and inferior longitudinal, verticalis, transversus) found towards the top of tongue control shape changes. Tongue muscles have distinctive fiber compositions,[23,24] which contribute to the functional abilities of the tongue to preserve airway patency. We propose that increased tongue fat not only increases the size of the tongue, which affects airway size and collapsibility, but also may adversely affect muscle function. Specifically, the increased

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presence of intramuscular fat may alter the tongue's shape and reduce its contractile force, affecting the tongue's ability to properly perform as a pharyngeal dilator muscle. We found a higher percentage of fat at the base of the tongue in apneics, the location where extrinsic muscles anchor the tongue to bone. This increased fat may affect the ability of each of the extrinsic muscles to properly position the tongue away from the airway.

The tongue is an important factor in mediating upper airway size and shape. The increased fat at the base of the tongue may alter the shape of the tongue in the retroglossal region, thereby reducing the size of the retroglossal airway and increasing the risk of sleep apnea. Airway shape has been shown to be an important in mediating airway closure during apnea.[25,26] Furthermore, changes in size and shape of the tongue (secondary to fat) may alter airway collapsibility and closing pressure (pcrit).

During sleep, interdigitated intrinsic and extrinsic muscles are co-activated in order to properly maintain the patency of the airway.[27] Increase in intramuscular fat may modify how forceful contractions are transmitted across multiple muscles within the tongue.[28] Fat infiltration at these critical junctions may affect the shape changes needed to prevent apneic events. Eckert et al. have shown that task failure in a tongue force fatigability test (maintenance of repetitive isometric contractions at 90% of maximal force protrusion) occurred more rapidly in apneics than controls.[29] Blumen et al. reported similar findings, indicating that apneics had longer recovery times between submaximal effort.[30] These examples of lowered resistance to fatigue in tongue endurance exercises may, at least in part, be due to the contribution of increased intramuscular fat in the apneic tongue. However, it should be noted that in the Eckert investigation maximal tongue protrusion force was greater in apneics than controls.[29] Tongue fat was not measured in this investigation, and the BMI of both the apneics and controls was lower than the BMI of our subjects.[29]

Relationship of Obesity to OSA

Obesity is the strongest risk factor for OSA, but the mechanisms underlying this relationship are not well understood. Weight loss has been shown to result in decreases in both fat and lean mass, although more than twice as much fat was lost compared to lean mass.[35] Weight gain has also been shown to result in a greater increase in fat than fat free tissue.[36,37] Thus, the relationship between obesity and OSA may be related to increases in both fatty and muscular tissue. The increase in muscular tissue with obesity may be secondary to fat infiltration within the muscle.

Parapharyngeal fat pads have also been shown to be enlarged in apneics and to contribute to airway narrowing.[38] However, other data has shown that the size of the fat pads were not statistically significantly different between apneics and normals after adjustment for craniofacial size, age, ethnicity, and gender.[14] Such data suggest that obesity compromises the upper airway in apneics through mechanisms other than fat deposition in the parapharyngeal fat pads. Using standard T1-weighted spin echo MRI, Li et al. have shown that apneics have increased deposition of fat within the soft palate compared to controls, independent of BMI.[20] We did not examine fat in the soft palate due to the difficulty in determining the boundary between the two muscles (tongue and soft palate) using Dixon images. Our data show that, independent of age, BMI, gender, and race, intramuscular tongue fat is increased in apneics compared to controls, providing a novel mechanism for explaining the relationship between obesity and OSA. We also showed that tongue fat is correlated with visceral abdominal fat and other fat deposits. It is not clear why the tongue has so much fat compared to the other upper airway muscles, but this fat deposition may be another form of visceral fat. Weight loss or upper airway exercises (which have been shown to improve OSA[39]) may decrease tongue fat. In the future, removal of tongue fat (via weight loss, upper airway exercises, or surgery) may be a potential treatment for OSA. Future studies examining such interventions are needed.

Study Limitations

There are several potential limitations of the present study that need to be addressed. Three-point Dixon MR images provide excellent separation of water and fat, although they are unable to clearly define tissue boundaries when adjacent tissues have similar tissue composition. This limitation was addressed by superimposing standard T1 spin-echo images on top of the Dixon MR images, which greatly improved tissue boundary visualization. The analysis of intramuscular fat was conducted using a thresholding method, which may be subject to over or under segmentation of fat. We believe the potential for this bias in our fat estimates is limited for several reasons. First, we observed excellent reproducibility (ICC > 0.95) and the analysis was completed by a single analyst. Second, all intramuscular fat measurements were completed using the same thresholding setting, and masseter fat percentages were found to be low, characteristic of normal muscle composition. Third, we were able to accurately quantify a known amount of fat in our phantom studies of hamburger and steak (Figure S2 and Table S1). Finally, our tongue fat percentages were comparable to those found in both the Nashi autopsy study (10% to 32%)[10] and Humbert's IDEAL-FSE (iterative decomposition of water and fat with echo asymmetry and least squares estimation - fast spin echo) imaging study (average tongue fat of 26.5%).[15]

Recruitment of obese controls (BMI $\geq$ 30 kg/m^2) aged 40-50 years is difficult, particularly for male controls, in part because OSA is highly prevalent in this demographic group. Therefore, we defined controls as those with an AHI $\leq$ 10 events/h, rather than using an AHI criterion of $\leq$ 5. There are several reasons why this cutpoint does not appear problematic. We scored the sleep studies using the alternative scoring method of the American Academy of Sleep Medicine,[40] which is a more liberal scoring system than the recommended criteria, potentially making the higher cutpoint more reasonable. Moreover, despite this higher cutpoint, the AHI in apneics was still much higher than in controls. Increasing the AHI cut-point for controls could have made it more difficult to find differences between apneics and controls; nonetheless, we found differences in tongue fat even with this cut-point for normals.

There was a noticeable, although not statistically significant, difference in the gender distribution between apneics (47% male) and controls (32% male). It is possible that this imbalance may have confounded our results. We addressed this potential confounding by not only adjusting for the effect of gender (in addition to age, BMI, and race) within our primary

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analysis, but also by repeating analyses within a subset of well-matched apneics and controls. Similar results were observed in this matched subsample, suggesting that covariate imbalance was not driving the observed result in the overall sample.

Since the goal of this study was to specifically examine tongue fat, we did not examine differences in other upper airway soft tissue structures or airway measures between the apneics and controls. However, we note that our group has published similar data in the past.[14] The difference in the total percentage of tongue fat between apneic and controls did not quite reach statistical significant after adjustment (P = 0.089). However, this difference was significant in the unadjusted models (P = 0.002), and there were statistically significant regional tongue fat percentage differences between apneics and controls.

We did not find a correlation between tongue volume and body mass index, which has been found in other studies.[41] This lack of correlation with BMI may be due to the restricted BMI range of the obese subjects recruited for this study. While specifically examining only obese patients may have limited our ability to observe strong correlations between BMI and tongue volumes, this was an essential aspect of the design in order to minimize the effect of BMI in primary comparisons between controls and apneics. Despite this limited BMI range, it should be noted that tongue fat was statistically significantly correlated with BMI.

Finally, we did not examine the effect of tongue fat on genioglossus activity, as this was not the purpose of the present study. However, we note that it is likely that tongue fat affects the mechanical function of the tongue. Therefore, such a study would be a logical future direction. As mentioned above, it is also of interest to examine the effect of weight loss and upper airway exercises on tongue fat.

CONCLUSIONS

We have combined spin echo and Dixon MR imaging into a novel paradigm to quantitatively assess fat deposits in the tongue, in an obese population with and without OSA. After adjusting for covariates (age, BMI, gender, and race), tongue volume and tongue fat were significantly enlarged in patients with OSA when compared to obese controls. We have shown this in our entire case-control sample and in our subsample matched specifically for age, BMI, gender, and race. We believe this increase in fat deposition not only enlarges tongue size, but also may decrease tongue force and hinder the tongue from properly functioning as an upper airway dilator muscle. Additionally, tongue size and tongue fat correlated with AHI. Further studies need to determine if weight loss decreases tongue fat, and whether improvements in sleep-disordered breathing are associated with changes in tongue fat.

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DISCLOSURE STATEMENT

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SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Polysomnography

Standard overnight sleep studies were performed, as described in our previous studies.[1] Polysomnograms were scored using the alternative criteria from the American Academy of Sleep Medicine (2007).[2] The AHI was calculated as the mean number of apnea and hypopnea events per hour of sleep. Obstructive apneas were defined as ≥ 90% drop in the thermal sensor excursion of baseline lasting at least 10 seconds; hypopneas were defined as a 50% reduction in airflow for > 10 seconds and associated with > 3% decrement in oxyhemoglobin saturation and/or an arousal. Nasal pressure monitors were used in all subjects to measure airflow. An event was called central when there was no associated chest wall movement. Mixed apneic events were scored as obstructive. Oxygen saturation was plotted across the time of night.

Upper Airway MRI Acquisition

High resolution upper airway MR imaging was performed on a 1.5 Tesla MAGNETOM Espree Scanner (Siemens Medical Systems, Malvern, PA) equipped with a prototype-enhanced gradient system. Patients were positioned supine with the head in a neutral anatomic position and secured using a head and neck coil,[3] this neutral position was defined by aligning the Frankfurt plane, a plane from the soft tissue orbit of the eye to the superior portion of the tragus of the ear, perpendicular to the scanning table. Subjects were instructed to breathe through the nose with the mouth closed and to refrain from swallowing during scanning. All MR scans were performed identically in both case and control subjects.

All images utilized an initial 9 second sagittal localizer scans (TR [repetition time] = 20 msec, TE [echo time] = 20 msec, 256 × 128 matrix, 1 NEX [number of signal averages]) in order to identify the boundaries of the nasopharynx and the larynx, which comprised the range of subsequent axial scans. Continuous 6.93 minute axial (TR = 500 msec, TE = 12 msec, 3 mm thick, Base Resolution = 256, Phase Resolution = 100%, Phase Oversampling = 60%, 256 × 128 matrix, 1 NEX) and 4.83 minute sagittal spin echo T1-weighted MR images (TR = 500

Table S1—Fat determinations in tissue phantoms.

Measured Hamburger Fat (mm^3) at Baseline	Measured Hamburger Fat (mm^3) after 3 cc of Injected Fat	Measure Steak Fat (mm^3) at Baseline	Measured Steak Fat (mm^3) after 6cc of Injected Fat
54.36	57.39	59.55	65.51

Table S2—Relationship between obesity and fat measurements in the tongue.

	BMI		Weight		Total Abdominal Fat		Subcutaneous Abdominal Fat		Visceral Abdominal Fat	
Primary Soft Tissue	**Rho**	**P**	**Rho**	**P**	**Rho**	**P**	**Rho**	**P**	**Rho**	**P**
Tongue, mm^3	0.06	0.5080	**0.26**	**0.0037**	0.02	0.8714	-0.16	0.0851	**0.34**	**0.0003**
Tongue fat, mm^3	**0.23**	**0.0102**	**0.27**	**0.0025**	**0.31**	**0.0009**	0.11	0.2325	**0.44**	**< 0.0001**
Tongue fat, %	**0.23**	**0.0105**	0.13	0.1627	**0.37**	**0.0001**	**0.28**	**0.0034**	**0.27**	**0.0047**

Significant Pearson's correlations are presented in bold.

Table S3—Comparison of tongue fat percentage and distribution within RP and RG regions in BMI, age, gender, and race-matched case-control pairs.

	Apneics (n = 18)		Controls (n = 18)			
Intramuscular Fat Percentage	**Mean**	**SD**	**Mean**	**SD**	**P†**	**P‡**
Total RP, %	24.7	9.1	21.5	8.5	0.308	0.308
Upper RP, %	2.8	2.8	3.8	4.8	0.257	0.159
Upper mid RP, %	14.9	10.2	15.5	9.5	0.875	0.871
Lower mid RP, %	30.0	11.8	23.2	9.0	**0.039**	**0.044**
Lower RP, %	35.4	13.9	30.5	12.6	0.283	0.276
Total RG, %	44.8	9.8	39.0	10.0	0.141	0.135
Upper RG, %	40.4	11.8	37.4	11.7	0.466	0.415
Upper mid RG, %	44.4	10.6	42.7	10.7	0.679	0.616
Lower mid RG, %	49.1	10.9	39.5	15.5	0.069	0.072
Lower RG, %	51.0	18.8	31.5	16.5	**0.007**	**0.010**

Significant differences are presented in bold. †P-value from paired *t*-test; ‡P-value from from regression analysis on difference in outcome, after adjustment for age and BMI differences; RP = retropalatal; RG = retroglossal.

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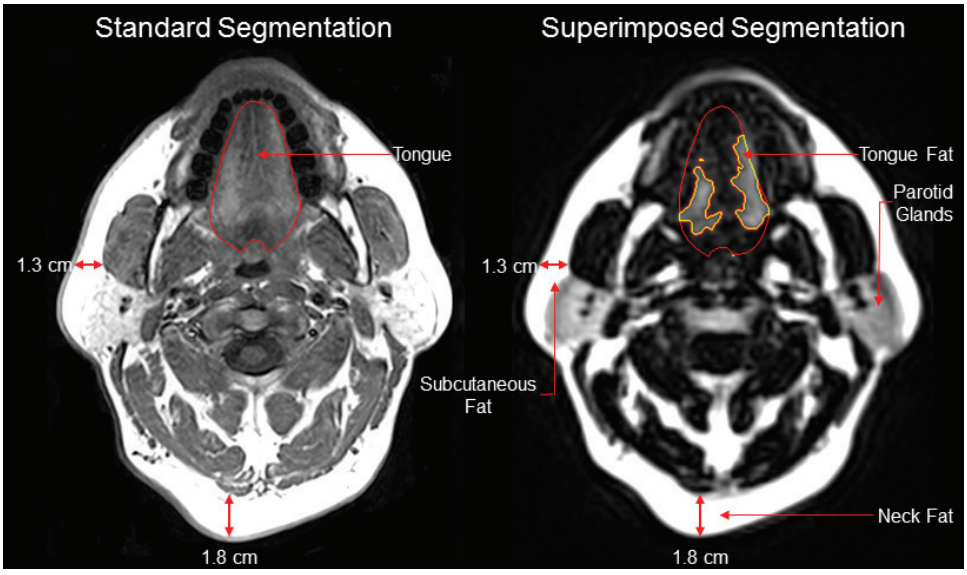

Figure S1—Axial standard T1-weight spin echo (left) and fat-weighted Dixon (right) MR images of the upper airway are shown above. Segmentation of the apneic tongue using standard T1-weighted spin echo MR image with superimposed segmentation on corresponding anatomic level of a Dixon image. Note that this correctly thresholded example shows comparable thicknesses of neck and subcutaneous fat within each image and visualization of parotid glands.

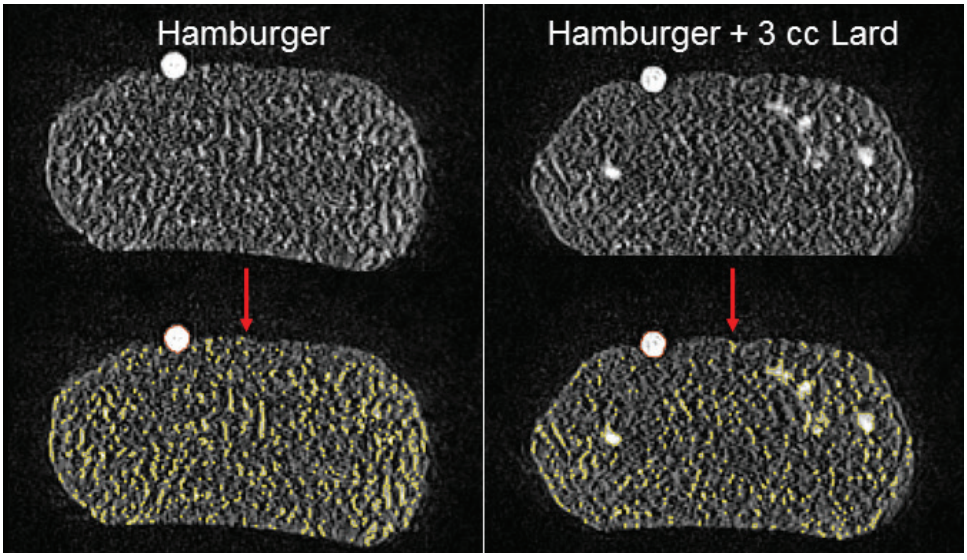

Figure S2—Coronal MR Dixon images of hamburger (left at baseline; right after 3 cc of lard were injected into same hamburger meat). Lard phantom is visible at top of hamburger, but water phantom is not visible (since Dixon MR images only highlight fat). Bright white areas on two right images indicate where fat was injected. Segmented Dixon MR images for fat (yellow) are shown below. Note that segmented hamburger with injected fat (bottom right image) includes large areas of injected fat.

msec, TE = 12 msec, 5 mm thick, Base Resolution = 256, Phase Resolution = 100%, Phase Oversampling = 13%, 256 × 128 matrix, 1 NEX) of the upper airway were acquired. The following 20 second T1-weighted gradient echo sequence (TR = 6.91 msec, 3 mm thick, Base Resolution = 128, Phase Resolution = 100%, Phase Oversampling = 0%, Slice Oversampling = 13%, TE_1 = 2.38 msec, TE_2 = 4.76 msec, 256 × 128 matrix, 1 NEX) was performed to obtain three-point Dixon MR images.

Anatomic Definitions, Measurements, and Analysis

Using Amira 4.1.2 (Visage Imaging, San Diego, CA), MR images of the upper airway were manually examined at the Pulmonary Imaging Center (University of Pennsylvania). The MRI analysis was split into three domains: airway measurements, volumetric analysis of soft tissues (tongue and masseter), and intramuscular fat (tongue and masseter) quantification. Airway measurements and soft tissue volumes were obtained from the standard T1 spin echo axial images. The segmented boundaries of the tongue and masseter were determined from the spin echo images and then superimposed on the Dixon MR images for proper tissue visualization. This method allows for direct measurement of intramuscular fat without segmenting the Dixon MR images. Fat-weighted Dixon MR images provide a clear indication of adipose tissue, but the boundaries of soft tissues are not as distinct in comparison to the standard spin echo images (Figure 1). Intramuscular fat was then determined using a thresholding method to differentiate fat from the rest of the tongue. The correct grayscale setting to segment tongue fat was chosen based on identifying the appropriate grayscale intensity of the surrounding subcutaneous and neck fat (Figure S1). The topographic distribution of tongue fat was further determined by subdividing the tongue into 8 sections, 4 within the retropalatal and 4 within the retroglossal regions of the tongue (see Figure 3 in manuscript). These sections were defined based on an equal number of axial slices (in each section) based on the craniocaudal axis from the midsagittal MRI, ranging from the upper and lower boundaries of the tongue. Fat within the masseter was examined as a comparable upper airway muscle using the same method and grayscale settings. A bilateral analysis of the masseter (N = 20) indicated no differences from a unilateral analysis. Therefore, fat percentage was determined from subjects' left masseter muscles. Subjects with major dental artifacts were excluded from the Dixon analysis. Dental artifacts can affect the mandible position by causing a protrusion of the bone into the boundaries of

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the tongue,[4] which in turn interferes with tongue fat quantification. All MR imaging analyses were performed by one trained technologist blinded to the subject's status (apneic or control) and supervised by a physician (RS). Reproducibility of this analysis was assessed by completing 10 analyses of randomized subjects in a single-blind trial on two separate occasions.

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Table S4—Relationship between AHI and regional tongue fat percentages.

Primary Soft Tissue	Partial Rho †	P
Total RP, %	0.01	0.952
Upper RP, %	-0.02	0.813
Upper mid RP, %	0.03	0.755
Lower mid RP, %	-0.01	0.900
Lower RP, %	0.07	0.458
Total RG, %	0.17	0.068
Upper RG, %	**0.20**	**0.029**
Upper mid RG, %	0.17	0.073
Lower mid RG, %	**0.20**	**0.033**
Lower RG, %	0.07	0.463

Significant correlations are presented in bold. † Adjusted for age, BMI, gender, and race.

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