Effect of Weight Loss on Upper Airway Anatomy and the Apnea Hypopnea Index: The Importance of Tongue Fat

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Scientific Knowledge This study examines the effect of weight loss on changes in tongue fat and other upper airway structures in individuals with obesity and sleep apnea assessed with Dixon magnetic resonance imaging. We also examined the influence of changes in upper airway structures with weight loss on improvements in apnea severity. Results indicate that tongue fat is decreased with weight loss and strongly associated with reductions in the apnea-hypopnea index.

What This Study Adds to this Field: This is the first study to show that weight loss decreases tongue fat in patients with sleep apnea, which explains one mechanism for the improvements in sleep apnea with reductions in weight. These findings suggest a potential unique therapeutic target for patients with obesity and sleep apnea, namely, reductions in tongue fat.

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ABSTRACT

Rationale: Obesity is the primary risk factor for sleep apnea (OSA). Tongue fat is increased in obese persons with OSA, and may explain the relationship between obesity and OSA. Weight loss improves OSA, but the mechanism is unknown.

Objectives: To determine the effect of weight loss on upper airway anatomy in subjects with obesity and OSA. We hypothesized that weight loss would decrease soft tissue volumes and tongue fat and these changes would correlate with reductions in apnea-hypopnea index (AHI).

Methods: Sixty-seven individuals with obesity and OSA (AHI≥10 events/hour) underwent a sleep study and upper airway and abdominal magnetic resonance imaging (MRI) before and after a weight loss intervention (intensive lifestyle modification or bariatric surgery). Airway sizes and soft tissue, tongue fat, and abdominal fat volumes were quantified. Associations between weight loss and changes in these structures, and relationships to AHI changes, were examined.

Measurements and Main Results: Weight loss was significantly associated with reductions in tongue fat, pterygoid and total lateral wall volumes. Reductions in tongue fat were strongly correlated with reductions in AHI (Pearson's rho=0.62, $p<0.0001$); results remained after controlling for weight loss (Pearson's rho=0.36, $p=0.014$). Reduction in tongue fat volume was the primary upper airway mediator of the relationship between weight loss and AHI improvement.

Conclusions: Weight loss reduced volumes of several upper airway soft tissues in subjects with obesity and OSA. Improved AHI with weight loss was mediated by reductions in tongue fat. New treatments that reduce tongue fat should be considered for patients with OSA.

Abbreviations: OSA = obstructive sleep apnea; AHI = apnea/hypopnea index; MRI = magnetic resonance imaging; BMI = body mass index

INTRODUCTION

Over 70% of adults in the United States are overweight (BMI [body mass index] >25 kg/m^2) or obese (BMI >30 kg/m^2) (1). Excess body weight is the primary risk factor for obstructive sleep apnea (OSA) (2-6), and studies support a relationship between weight change and OSA risk or severity (6-8). Weight loss is recommended for the treatment of OSA (9). Across multiple studies, weight losses secondary to a range of dietary and lifestyle modifications are associated with significant reductions in the apnea hypopnea index (AHI) (3, 6-8, 10-15). For example, in the Wisconsin Sleep Cohort, a 1% increase/decrease in body weight was associated with a corresponding 3% increase/decrease in AHI and a 10% weight gain was associated with 6-fold increased risk of developing an AHI >15 events/hour (6). Systematic reviews and meta-analyses of surgical (14) and non-surgical (15) weight loss support this relationship. However, while OSA severity is generally improved, OSA may not be eliminated after weight loss. Why obesity is associated with OSA, why weight loss improves OSA, and why weight gain exacerbates OSA remain unanswered fundamental questions. Addressing these questions is important for optimizing clinical management and personalized OSA treatments.

The mechanisms for improved AHI with weight loss are not well understood. Evidence suggests weight loss may differentially affect AHI in the supine versus non-supine positions (17). Decreased size of the upper airway and larger upper airway soft tissue volumes increase risk for OSA (18). Changes in these anatomical factors with weight loss may explain the improvements in AHI. Our group has shown that tongue fat is increased in obese patients with OSA, compared to obese patients without OSA (19). Thus, reduced tongue fat with weight loss may reduce OSA severity. Similar relationships may exist for other upper airway anatomy implicated in OSA risk (18).

The primary goal of this study was to evaluate changes in upper airway anatomy with weight loss in patients with OSA, and to understand how these changes relate to improvements in AHI. We hypothesized that weight loss would result in corresponding changes in upper airway anatomy (e.g., increased airway size and decreased soft tissue volumes), and these changes, particularly decreased tongue fat, would associate with decreased OSA severity.

METHODS

See details (**Online Supplement)**.

Subjects

This study included subjects with obesity and OSA presenting for bariatric surgery or lifestyle modification intervention for weight loss, recruited from the Penn Center for Sleep Disorders, the University of Pennsylvania's Bariatric Surgery Program, or the Center for Weight and Eating Disorders. The study was approved by the University of Pennsylvania Institutional Review Board. Written informed consent was obtained from all participants. Participants were >18 years-old with an AHI≥10 events/hour on polysomnography (19). Exclusion criteria included inability to undergo magnetic resonance imaging (MRI) or pregnancy. MRI and polysomnography were performed before weight loss treatment and repeated after 6 months.

Weight Loss Protocols

Participants underwent lifestyle modification for weight loss (n=49) or bariatric surgery (n=18; gastric sleeve [n=8], bypass (Roux-en-Y) [n=9] or banding [n=1]). The lifestyle modification was based upon the Diabetes Prevention Program and designed to promote weight loss of 5-10% through caloric restriction, increased physical activity, and behavioral modifications (20).

Polysomnography

Standard polysomnography was conducted as previously described (18, 19).

Magnetic Resonance Imaging

MRI studies were acquired using a 1.5 Tesla MAGNETOM Espree scanner (Siemens Medical Systems, Malvern, PA) as previously described (18, 19, 21, 22). Amira 4.1.2 analysis software (Visage Imaging, San Diego, CA) was utilized to quantify MRI measures in 3 domains: *Airway Sizes* (10 measures) – airway volume, average cross-sectional area, minimum airway area, minimum anterior-posterior distance and minimum lateral distance in the retropalatal (RP) and retroglossal (RG) regions; *Soft Tissue Volumes* (12 measures) – tongue, tongue fat, soft palate, parapharyngeal fat pads, lateral walls, pterygoids, epiglottis, and combined soft tissue volume (the sum of these structures); and *Abdominal Fat Volumes* (3 measures) – total, subcutaneous and visceral fat volumes.

Reproducibility Assessment

Reproducibility of measurements was assessed by calculating intraclass correlation coefficients (ICCs) from data quantified on separate MRIs performed six months apart in a sample of 17 weight stable individuals (defined as follow-up weight within 2.5% of baseline weight). This sample was chosen to represent individuals that did not undergo weight loss intervention. ICCs quantify reproducibility as *poor* (<0.00), *slight* (0.00-0.20), *fair* (0.21-0.40), *moderate* (0.41-0.60), *substantial* (0.61-0.80) and *almost perfect* (0.81-1.00) (23).

Statistical Analysis

Analyses were performed using Stata, Version 14 (StataCorp LP, College Station, TX), SAS Version 9.4 (SAS Institute Inc., Cary, NC) and SPSS 24 (IBM Corp., Armonk, NY). Changes scores were calculated as follow-up minus baseline. Relationships between weight loss and anatomic changes were assessed using unadjusted Pearson's linear correlations and partial

Pearson's correlations adjusted for baseline age, sex, race, height and AHI. Associations between anatomy changes and AHI change were performed similarly. Complementary analyses comparing patients that lost ≥2.5% weight and those with stable/increased weight were conducted using T-tests and linear regression adjusted for age, sex, race, height, AHI and baseline MRI measure (absolute changes only). Within group changes were assessed with paired T-tests. A domain-specific Hochberg step-up correction (24-26) was used to control for multiple comparisons (see **Online Supplement**); p<0.05 was considered nominally significant. Mediators between percent changes in weight and AHI were evaluated using conditional process analysis (27, 28) (**Figure S1**). Bias-corrected 95% confidence intervals (CIs) were estimated via bootstrapping to verify indirect (mediating) effects; mediation was shown if the CI excluded zero (27, 29).

RESULTS

Measurement Reproducibility

To assess measurement reproducibility, we calculated ICCs using data from MRIs taken six months apart in a sample of weight-stable apneic and non-apneic patients (**Table S1**). Nearly all measurements demonstrated substantial (ICC between 0.61-0.80) or almost perfect (ICC between 0.81-1.00) reproducibility. Fat pad volume (ICC=0.353) and both total (ICC=0.489) and subcutaneous (ICC=0.536) abdominal fat volumes showed fair to moderately reproducible.

Participants Characteristics

Sixty-seven patients with OSA were included (**Table 1**). Participants were middle-aged (49.4±11.9 years) and obese (BMI of 42.6±8.5 kg/m^2), 40.3% were male and 47.0% were Caucasian. Overall, participants lost 9.5±10.8% of their body weight (p<0.0001) and AHI improved by 30.7±66.7% (p=0.0004). Apneics who lost ≥2.5% weight had a significant AHI reduction (-23.3±21.9; p<0.0001), compared to no change in those that did not (p=0.856) (see **Online Supplement**).

Changes in Anatomy with Weight Loss

To understand how weight loss affected the upper airway and abdominal fat, we assessed Pearson's correlations between percent changes in weight and anatomical structures (**Table 2: A-C**). Analyses of absolute changes are presented in **Table S2 (A-C)**. Comparisons of patients that lost ≥2.5% weight and those that were weight stable or gained weight are detailed in the Online Supplement (see **Tables S3 and S4: A-C**).

Airway Sizes

Table 2A shows associations between changes in weight and airway sizes. Larger percent decreases in weight were significantly associated with greater percent increases in RG minimum area (Pearson's partial rho=-0.43, p=0.001), controlling for covariates. Significant or nominal correlations were also observed with changes in the shape of the RP airway (**Table 2A**). Weight loss was associated with decreased AP distance (partial rho=0.36, p=0.006) and increased lateral distance (partial rho=-0.34, p=0.009) in the RP region. Similar effects on RP airway shape, but not RG minimum area, were observed for absolute changes (**Table S2A**). Comparisons between weight loss groups are shown in **Table S3A** and **S4A** (detailed in the Online Supplement). RP airway sizes and RG minimum area increased in patients that lost weight; changes in RP minimum lateral distance and RG minimum area were different between those that lost weight and those that did not.

Soft Tissue Volumes

Table 2B details correlations between percent changes in weight and soft tissue volumes. In adjusted analyses, larger percent reductions in weight were significantly correlated with greater percent reductions in tongue fat (partial rho=0.62, p<0.0001), pterygoid (partial rho=0.40, p=0.002) and total lateral wall (partial rho=0.40, p=0.002) volumes, and nominally correlated with greater percent reductions in RP lateral wall volume (partial rho=0.31, p=0.017). The relationship between reduction in tongue fat volume and percentage change in weight is illustrated in **Figure 1**. Similar results were observed for absolute changes (**Table S2B**), with correlations between absolute weight change and change in tongue fat (partial rho=0.48, p=0.001), pterygoid (partial rho=0.37, p=0.005) and total lateral wall (partial rho=0.28, p=0.035) volumes. Most soft tissue measures showed significant decreases among patients with OSA who lost weight (**Online Supplement, Tables S3B and S4B**), and there were significant differences in tongue fat volume, pterygoid volume and total lateral wall volume changes between weight loss groups. Changes in soft tissue volumes and tongue fat are illustrated in **Figures 2 and 3**.

Abdominal Fat Volumes

Table 2C shows relationships between changes in weight and abdominal fat. Strong positive correlations were observed between percent change in weight and percent changes in total (partial rho=0.54, p=0.0001), subcutaneous (partial rho=0.52, p=0.0003) and visceral (partial rho=0.49, p=0.001) abdominal fat in adjusted analyses. Similar associations were seen for absolute changes (**Table S2C**). There were larger reductions in each measurement among those who lost weight, compared to no change in those who did not (**Tables S3C and S4C**). The percentage change in visceral fat was greater than the percentage change in subcutaneous fat among participants who lost weight (p=0.002). Changes in abdominal fat are illustrated in **Figure 4**.

Associations between Changes in Tongue Fat and Abdominal Fat

We assessed correlations between tongue fat and abdominal fat changes, given that both associated with percent changes in weight. Results are detailed in the **Online Supplement**.

Associations between Changes in Anatomy and Changes in AHI

To understand the relationship between changes in upper airway anatomy and OSA severity, we evaluated correlations with percentage changes in AHI (**Tables 3A-C**). Larger percentage reductions in weight were strongly correlated with greater reductions in AHI (partial rho=0.68, p<0.0001). Complementary analyses with absolute changes are presented in **Tables S5 A-C**. Given evidence of positional differences in the AHI response to weight loss (17), analyses examining correlations with changes in supine and non-supine AHI are presented in the **Online Supplement** (**Tables S6 A-C**), among patients with positional AHI≥5 events/hour at baseline. Percentage reductions in weight were strongly correlated with non-supine AHI (partial rho=0.63, p<0.0001), but not supine AHI (partial rho=-0.06, p=0.753).

Airway Sizes

When evaluating the effect of changes in airway size on the AHI (**Table 3A**), controlling for covariates, greater reductions in RP minimum AP distance (partial rho=0.30, p=0.022) and increases in RP minimum lateral distance (partial rho=-0.32, p=0.015) were nominally associated with greater decreases in AHI. Thus, changes in the shape of the RP airway affect OSA severity. In the retroglossal region, larger increases in the minimum area were associated with greater AHI reductions (partial rho=-0.35, p=0.008). These correlations became non-significant after correction for multiple comparisons. There were no significant correlations with absolute changes (**Table S5A**) or positional AHI (**Table S6A**).

Soft Tissue Volumes

Among soft tissue measures (**Table 3B**), greater percentage decreases in tongue fat were associated with larger reductions in AHI (partial rho=0.62, p<0.0001), controlling for clinical covariates. This result remained nominally significant also controlling for weight change (partial rho=0.36, p=0.014), suggesting reduced tongue fat is independently associated with reduced AHI (**Figure 1**). Reductions in RP lateral wall volume were nominally correlated with reductions in AHI (partial rho=0.32, p=0.014); results were not significant controlling for change in weight. Associations between absolute changes in tongue fat and AHI were also observed (**Table S5B**). Percentage reduction in tongue fat was more strongly correlated with reductions in non-supine AHI (partial rho=0.59, p=0.0004) than supine AHI (partial rho=0.22, p=0.260) (**Table S6B**).

Abdominal Fat Volumes

In adjusted analyses, we observed significant correlations between reductions in AHI and reductions in total (partial rho=0.38, p=0.009), subcutaneous (partial rho=0.39, p=0.008) and visceral (partial rho=0.31, p=0.039) abdominal fat (**Table 3C**). Unlike tongue fat, correlations were non-significant controlling for change in weight. Similar results were found for absolute changes (**Table S5C**). Correlations were similar for supine and non-supine AHI, but not significant in the smaller sample (**Table S6C**).

Mediation Analyses

We next evaluated whether changes in specific anatomical structures mediate the relationship between percentage change in weight and percentage change in AHI. In our patients, each 1% change in weight was associated with a corresponding 4% change in AHI (unstandardized total effect [95% CI] = 3.98 [2.74, 5.22]; standardized total effect = 0.648). Percent change in airway size or abdominal fat volumes did not significantly mediate this relationship (**Table 4**). On the other hand, analyses of soft tissue volumes indicated that percent reduction in tongue fat volume was a significant individual mediator between percent change in weight and AHI (unstandardized indirect effect [95% CI] = 1.255 [0.238, 2.572]; standardized indirect effect =

0.225; **Table 4**). Changes in tongue fat accounted for ~30% of the total effect of weight loss on AHI improvement (**Figure 5**). No other soft tissues were significant individual mediators. Thus, these results indicate that change in tongue fat volume is the primary upper airway mediator of the relationship between weight change and change in AHI.

DISCUSSION

This study of patients with obesity and OSA undergoing lifestyle modification or bariatric surgery is the first to show that weight loss decreases tongue fat and the reduction in tongue fat is a mediator of the improvement in AHI. Primary findings include: 1) weight loss was significantly associated with reduced tongue fat volume, pterygoid volume and total lateral wall volume; 2) strong correlations were observed between reductions in tongue fat volume and reductions in AHI; and 3) reduction in tongue fat volume was the primary upper airway mediator of the relationship between reductions in weight and AHI. Beyond providing important mechanistic insights, these results suggest tongue fat could be a potential new target for OSA therapy.

Effect of Weight Loss on Upper Airway Caliber and Surrounding Soft Tissues

Several upper airway measurements changed with weight loss, including retropalatal airway shape and volumes of tongue fat, pterygoid, and the lateral walls. However, other upper airway measurements showed no changes. Thus, weight loss may differentially effect upper airway anatomy; the pathogenesis of this is unclear, but could be genetically determined.

Weight loss reduces adipose tissue volume. Thus, the reduction in tongue fat was expected. However, reductions were also observed for the lateral walls and pterygoid, both of which do not contain fat deposits observable with MRI (although intramyocellular lipid droplets have been observed in the pharyngeal constrictors that make up the lateral walls using electron microscopy) (30). The mechanisms by which weight loss affects soft tissues likely differs for tissues that are primarily fat versus relatively fat-free (31-33). Analyses have shown reduced muscle mass and volume with weight loss (33-37), which could account for reductions in pterygoid and lateral wall volumes. The change in lateral wall volume may be due to other mechanisms, including reduced size of the parapharyngeal fat pads (providing space for the lateral walls) and/or tracheal tug (putting tension and thereby narrowing the lateral walls) secondary to improved lung volume with weight loss.

The repeated trauma of obstruction with OSA may also trigger an edematous response (38). As OSA improves with weight loss, the trauma becomes less severe, reducing the inflammation of pharyngeal tissues. While this would explain the reduction in lateral wall and pterygoid volumes, it does not account for the lack of change in soft palate volume. Since the soft palate contains fat (39), high resolution Dixon imaging (19) may be required to detect fat-specific effects, as seen in the tongue. Alternatively, the soft palate has been shown to be inflamed and fibrosed in apneics (40) and weight loss should not reverse fibrosis. Ultimately, differences in the changes of distinct upper airway soft tissues with weight loss underscores the complexity of these relationships. Understanding the reasons for these differences may provide insight into OSA heterogeneity, and inform personalized treatments.

Tongue Fat as a Potential Therapeutic Target

This study observed strong correlations between tongue fat reduction and improvement in AHI, and mediation analyses supported changes in tongue fat as the primary upper airway mediator between weight loss and AHI reduction. Although the mechanism for this relationship is unknown, reduction in tongue fat affects tongue size and may increase upper airway caliber or improve tongue function. In particular, fat can infiltrate the muscle bundles and affect muscle strength and obesity adversely affects muscle function, with inverse relationships between muscle lipid content and muscle force, velocity, and power (30, 33, 41, 42). Thus, reduced tongue fat should improve muscle function and could prevent collapsibility during sleep. Regardless of mechanism, our results underscore the potential efficacy of OSA therapies that reduce tongue fat.

Although not directly studied, several potential therapies exist. Dixon MRI before and after these interventions is a logical step to determine feasibility and efficacy. Upper airway exercises improve OSA and reduce AHI (43-45); reduced tongue fat is one potential mechanism. Tongue fat may differentially respond to weight loss approaches that vary in dietary composition, although this remains to be investigated. Cold therapies could also potentially remove tongue fat. For example, cryolipolysis is a non-invasive cooling technique that lyses adipocytes and is effective and safe for reducing abdominal and submental fat (46, 47); a similar technique may reduce tongue fat. While experimental, our data provide the foundation for investigation of these therapies through animal or human studies.

Our study may also explain why upper airway surgery is not more effective in treating OSA. Coblation has been used to treat patients with OSA by reducing tongue size, however, it has limited efficacy (48, 49). Coblation does not discriminate between muscle and fat, but instead uses radiofrequency and water to generate a plasma that vaporizes all soft tissue types. Thus, our results demonstrating a specific role for tongue fat volume could explain the observed lack of efficacy. If only fat tissue was removed, coblation could be more effective. Future studies are warranted to study this.

Measurement Reproducibility

Our results confirm reproducibility of MRI measurements at two time points. Nearly all measures showed substantial or almost perfect reproducibility within weight stable individuals. Moderate-to-fair reproducibility was observed for fat pad volume, as well as total and subcutaneous abdominal fat. The likely explanation for this comparatively lower reproducibility is related to lumbar flexion/extension (which is difficult to control) and its effect on the L4-L5 junction, which is the inferior boundary for the abdominal fat measurements. Depending on the amount of lumbar flexion/extension the region of interest analyzed may be different by 1 MRI slice, which can alter the quantitative abdominal fat measures. Nonetheless our results are consistent with our prior studies demonstrating the validity and reproducibility of volumetric measurements quantified by MRI. Previously (21), we demonstrated the accuracy of volumetric measures against a phantom of known volume, the reliability of analyses on the same images, and high reproducibility on repeated MRI taken approximately one month apart. Similarly, our study on tongue fat demonstrated high reproducibility of the measurement technique (19). Moreover, our previous study on abdominal fat measurements demonstrated high reproducibility across multiple raters on repeated images (22). Thus, results from the present study extend evidence of reproducibility to a six month follow-up period in a weight stable population.

Limitations

One limitation of utilizing MRI at multiple timepoints is that anatomic changes may reflect MRI variability (e.g., differences in head and/or neck position), rather than solely the effect of weight loss. The use of standardized protocols and observation that nearly all upper airway measurements showed substantial or almost perfect reproducibility in weight stable patients mitigates this concern. Including medical and surgical weight loss could be viewed as a limitation. However, utilizing multiple types of weight loss interventions increased variability in weight changes (augmenting statistical power) and improves generalizability. Ultimately, we were able to show changes to the upper airway soft tissues and abdominal fat across different treatments and weight loss amounts. However, six months may not be enough time for all structures to show meaningful changes; longer studies should be conducted.

The lack of significant mediation of airway size on the relationship between weight change and AHI may reflect difficulties in measuring airway caliber with MRI during wakefulness. Airway caliber changes during inspiration and expiration (50, 51) but our imaging sequences were performed over several minutes, resulting in average measurements of airway size. Averages may not be sensitive enough to detect mediating effects. In future studies, dynamic MRI should be performed to better capture the effect of awake airway dimensional changes with weight loss.

Conclusions

This study is the first to use volumetric MRI to examine changes in airway sizes, pharyngeal soft tissues and abdominal fat with weight loss in persons with obesity and OSA. We confirmed that our analysis techniques are a reliable means of quantifying the size of upper airway structures over a six month period. Weight loss reduced adipose tissue volumes in the abdomen and upper airway (in particular tongue fat), as well as volumes of soft tissues consisting primarily of fat-free mass (lateral walls, pterygoid). Analyses indicated that reduction in tongue fat was the primary upper airway mediator of the relationship between reductions in weight and AHI. These results elucidate, in part, the mechanism by which weight loss improves OSA and provide targets for potential new therapies in lieu of weight loss.

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Figure Legends

Figure 1. *The relationship of percentage change in tongue fat volume with percentage change in weight and AHI.* The associations between the percentage change in tongue fat and weight loss (left panel) and AHI change (right panel) are illustrated among obese apneics undergoing surgical or medical weight loss. Strong positive correlations were observed between tongue fat change and both measures (Pearson's partial rhos = 0.62, $p<0.0001$), in covariate adjusted analyses. Mediation analyses suggest that percentage change in tongue fat was the primary upper airway mediator between percentage weight loss and percentage reductions in AHI.

Figure 2. *Changes in upper airway soft tissue structures with weight loss.* Three-dimensional reconstructions derived from axial MRI (T1-weighted, spin echo, 3 mm slice thickness), demonstrating changes in selected upper airway soft tissue structures between baseline and 6 month follow-up in a male patient with sleep apnea. Structures include: tongue, defined as the genioglossus muscle (red); soft palate (magenta); parapharyngeal fat pads (yellow); and lateral pharyngeal walls (green). The region of interest extends from the superior appearance of the tongue to the appearance of the hyoid bone.

Figure 3. *Change in tongue fat volume with weight loss.* Three-dimensional reconstruction of tongue (red) and tongue fat (yellow) derived from axial MRI (T1-weighted, spin echo, 3 mm slice thickness) and Dixon fat-only MRI (3 mm slice thickness), demonstrating loss of tongue fat between baseline and a 6 month follow-up visit in the same male apneic as shown in **Figure 2**. The tongue is defined as the genioglossus muscle, and tongue fat is defined as all fat within the genioglossus.

Figure 4. *Change in abdominal fat volumes with weight loss.* Three-dimensional reconstructions of abdomen derived from axial MRI (T1-weighted, spin echo, 10 mm slice thickness) showing fat loss between baseline and a 6 month follow-up visit in the same male apneic as shown in **Figures 2 and 3**. Subcutaneous fat (cyan), visceral fat (yellow), and the liver (red) have been highlighted. Subcutaneous fat is defined as all fat superficial to the abdominal fascia. Visceral fat is defined as all fat within the abdominal fascia that is not part of the spinal column. The region of interest extends from the superior appearance of the liver to the L5-S1 intervertebral disc.

Figure 5. *Mediation of percentage change in weight and AHI by percentage change in tongue fat.* Results of the single mediator model of percent change in tongue fat volume mediating the relationship between percent change in weight and in AHI are shown. Unstandardized path coefficients, interpreted as the expected percentage change in outcome for a 1 percentage change in predictor, of the relationships between percent weight change and percent change in tongue fat (_path a = 1.168***_), between percent change in tongue fat and percent change in AHI (_path b = 1.074*_), and the remaining direct effect between percent change in weight and percent change in AHI (_path c' = 2.337**_) are also shown. Significance of path coefficients is denoted as: *p<0.05, **p<0.01, ***p<0.001.

Table 1: Demographics of the study sample, overall and by weight loss group

Variable	All Participants	Weight Stable/Gain[†]	Weight Loss[†]	p[‡]
N	67	20	47	–
Age, years	49.4 ± 11.9	51.1 ± 11.8	48.7 ± 12.0	0.472
Male, %	40.3%	35.0%	42.6%	0.564
White, %	47.0%	40.0%	50.0%	0.454
Height, inches	67.1 ± 4.3	66.9 ± 4.6	67.1 ± 4.2	0.814
Weight, pounds				
Baseline	272.0 ± 55.7	287.4 ± 61.7	265.5 ± 52.3	0.143
Follow-up	244.9 ± 54.2	292.9 ± 58.4	224.4 ± 37.1	0.0001
Change	-27.1 ± 33.0[§]	5.6 ± 11.6[§]	-41.0 ± 29.1[§]	<0.0001
% Change	-9.5 ± 10.8[§]	2.3 ± 4.5[§]	-14.5 ± 8.5[§]	<0.0001
BMI, kg/m^2				
Baseline	42.6 ± 8.5	45.3 ± 8.8	41.5 ± 8.2	0.099
Follow-up	38.4 ± 8.5	46.2 ± 8.7	35.1 ± 5.8	<0.0001
Change	-4.2 ± 5.2[§]	0.9 ± 2.0[§]	-6.4 ± 4.5[§]	<0.0001
% Change	-9.5 ± 10.8[§]	2.3 ± 4.5[§]	-14.5 ± 8.5[§]	<0.0001
AHI, events/hour				
Baseline	40.8 ± 28.6	39.2 ± 31.5	41.4 ± 27.6	0.776
Follow-up	25.1 ± 23.6	40.1 ± 24.7	18.6 ± 20.0	0.0004
Change	-16.0 ± 24.5[§]	0.9 ± 21.9	-23.3 ± 21.9[§]	0.0001

[†]Weight loss defined as ≥2.5% decrease in weight from baseline and stable/gain defined as <2.5% decrease in weight; [‡]p-value from T-test or chi-squared test comparing values between weight loss and stable/gain groups; [‡]within group change significantly different from zero (p<0.05) in paired T-test.

Table 2A: Pearson's Correlations between Percent Change in Weight and Percent Change in Airway Dimensions among Patients with OSA

Airway Sizes	Unadjusted			Adjusted		
	N	rho†	p	N	rho‡	p
RP Airway Volume	64	-0.05	0.668	63	-0.09	0.502
RP Cross Sectional Area	64	-0.14	0.285	63	-0.18	0.179
RP Minimum Area	64	0.01	0.942	63	0.01	0.936
RP Minimum AP Distance	64	0.30	0.017	**63**	**0.36**	**0.006**
RP Minimum Lateral Distance	64	-0.26	0.035	63	-0.34	0.009
RG Airway Volume	62	0.08	0.523	61	0.10	0.443
RG Cross Sectional Area	60	0.16	0.224	59	0.14	0.321
RG Minimum Area	61	-0.33	0.008	**60**	**-0.43**	**0.001**
RG Minimum AP Distance	60	0.15	0.253	59	0.12	0.385
RG Minimum Lateral Distance	60	-0.02	0.875	59	-0.05	0.701

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race, AHI and height. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table 2B: Pearson's Correlations between Percent Change in Weight and Percent Change in Soft Tissue Volumes among Patients with OSA

Soft Tissue Volumes	Unadjusted			Adjusted		
	N	rho†	p	N	rho‡	p
Combined Soft Tissue	63	0.14	0.268	62	0.18	0.187
Soft Palate	64	-0.15	0.234	63	-0.14	0.303
Genioglossus	64	-0.02	0.866	63	-0.01	0.937
Other Tongue	64	0.01	0.962	63	0.04	0.788
Tongue Fat	**52**	**0.62**	**<0.0001**	**51**	**0.62**	**<0.0001**
Total Tongue	64	-0.03	0.833	63	0.00	0.993
Epiglottis	63	0.07	0.591	62	0.09	0.486
Fat Pads	63	0.08	0.529	62	0.08	0.536
Pterygoid	**64**	**0.42**	**0.001**	**63**	**0.40**	**0.002**
RP Lateral Walls	64	0.25	0.044	63	0.31	0.017
RG Lateral Walls	64	0.19	0.137	63	0.23	0.088
Total Lateral Walls	64	0.32	0.011	**63**	**0.40**	**0.002**

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race, AHI and height. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table 2C: Correlations between Percent Change in Weight and Percent Change in Abdominal Fat Measures among Patients with OSA

Abdominal Fat Volume	Unadjusted			Adjusted†		
	N	rho†	p	N	rho‡	p
Abdominal Total Fat	**51**	**0.52**	**0.0001**	**50**	**0.54**	**0.0001**
Abdominal Subcutaneous Fat	**51**	**0.50**	**0.0002**	**50**	**0.52**	**0.0003**
Abdominal Visceral Fat	**51**	**0.46**	**0.001**	**50**	**0.49**	**0.001**

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race, AHI and height. Significant values after Hochberg correction shown in **bold**.

Table 3A: Pearson's correlations between Percent Change in AHI and Percent Change in Airway Size among Patients with OSA

Airway Sizes	Unadjusted			Adjusted Results: Covariates Only			Adjusted Results: Covariates and Weight Change		
	N	**rho†**	**p**	**N**	**rho‡**	**p**	**N**	**rho§**	**p**
RP Airway Volume	63	0.13	0.318	62	0.10	0.453	62	0.21	0.124
RP Cross Sectional Area	63	0.07	0.562	62	0.03	0.817	62	0.19	0.155
RP Minimum Area	63	0.02	0.904	62	-0.01	0.946	62	-0.02	0.862
RP Minimum AP Distance	63	0.25	0.045	62	0.30	0.022	62	0.09	0.494
RP Minimum Lateral Distance	63	-0.23	0.076	62	-0.32	0.015	62	-0.14	0.304
RG Airway Volume	61	0.11	0.396	60	0.08	0.558	60	0.03	0.804
RG Cross Sectional Area	59	0.34	0.009	58	0.30	0.025	58	0.28	0.039
RG Minimum Area	60	-0.28	0.033	59	-0.35	0.008	59	-0.11	0.428
RG Minimum AP Distance	59	0.18	0.175	58	0.16	0.233	58	0.09	0.512
RG Minimum Lateral Distance	59	0.10	0.470	58	0.04	0.753	58	0.11	0.428

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table 3B: Pearson's Correlations between Percent Change in AHI and Percent Change in Soft Tissues among Patients with OSA

Soft Tissue Volumes	Unadjusted			Adjusted Results: Covariates Only			Adjusted Results: Covariates and Weight Change		
	N	**rho†**	**p**	**N**	**rho‡**	**p**	**N**	**rho§**	**p**
Combined Soft Tissue	62	0.19	0.139	61	0.23	0.090	61	0.17	0.204
Soft Palate	63	-0.16	0.202	62	-0.14	0.305	62	-0.06	0.664
Genioglossus	63	0.13	0.324	62	0.16	0.228	62	0.23	0.082
Other Tongue	63	0.07	0.577	62	0.11	0.401	62	0.13	0.343
Tongue Fat	**51**	**0.61**	**<0.0001**	**50**	**0.62**	**<0.0001**	50	0.36	0.014
Total Tongue	63	0.12	0.340	62	0.18	0.185	62	0.25	0.063
Epiglottis	62	0.05	0.687	61	0.10	0.459	61	0.05	0.712
Fat Pads	62	0.14	0.278	61	0.15	0.281	61	0.12	0.364
Pterygoid	63	0.24	0.055	62	0.19	0.145	62	-0.09	0.488
RP Lateral Walls	63	0.26	0.037	62	0.32	0.014	62	0.18	0.189
RG Lateral Walls	63	0.07	0.600	62	0.04	0.791	62	-0.12	0.357
Total Lateral Walls	63	0.24	0.055	62	0.26	0.051	62	0.03	0.813

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table 3C: Pearson's Correlations between Percent Change in AHI and Percent Change in Abdominal Fat Measures among Patients with OSA

Abdominal Fat Volume	Unadjusted			Adjusted Results: Covariates Only			Adjusted Results: Covariates and Weight Change		
	N	**rho†**	**p**	**N**	**rho‡**	**p**	**N**	**rho§**	**p**
Abdominal Total Fat	**50**	**0.37**	**0.008**	**49**	**0.38**	**0.009**	49	-0.04	0.790
Abdominal Subcutaneous Fat	**50**	**0.38**	**0.006**	**49**	**0.39**	**0.008**	49	0.00	0.993
Abdominal Visceral Fat	**50**	**0.29**	**0.041**	**49**	**0.31**	**0.040**	49	-0.11	0.466

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction shown in **bold**.

Table 4: Single mediator modeling results evaluating percent changes in airway, soft tissue and abdominal fat as mediators of the relationship between percent change in weight and AHI

Domain/Measure	Indirect Effect (Bias-Corrected 95% CI)†,‡: Standardized	Indirect Effect (Bias-Corrected 95% CI)†,‡: Unstandardized
Airway Sizes		
RP Airway Volume	-0.013 (-0.101, 0.025)	-0.082 (-0.559, 0.157)
RP Average Airway Area Per Slice	-0.025 (-0.116, 0.019)	-0.154 (-0.656, 0.117)
RP Minimum Area	-0.0002 (-0.025, 0.018)	-0.002 (-0.156, 0.107)
RP Minimum AP Distance	0.027 (-0.052, 0.189)	0.166 (-0.311, 1.270)
RP Minimum Lateral Distance	0.037 (-0.033, 0.146)	0.229 (-0.194, 0.913)
RG Airway Volume	0.002 (-0.034, 0.032)	0.013 (-0.205, 0.188)
RG Average Airway Area Per Slice	0.030 (-0.040, 0.120)	0.184 (-0.230, 0.814)
RG Minimum Area	0.040 (-0.060, 0.133)	0.246 (-0.405, 0.667)
RG Minimum AP Distance	0.011 (-0.030, 0.048)	0.065 (-0.180, 0.285)
RG Minimum Lateral Distance	-0.006 (-0.048, 0.023)	-0.034 (-0.296, 0.137)
Soft Tissue Volumes		
Combined Soft Tissues	0.020 (-0.041, 0.057)	0.122 (-0.249, 0.330)
Soft Palate	0.006 (-0.036, 0.039)	0.039 (-0.209, 0.247)
Genioglossus	-0.004 (-0.072, 0.036)	-0.026 (-0.426, 0.235)
Other Tongue	0.002 (-0.053, 0.030)	0.014 (-0.333, 0.170)
Tongue Fat	**0.225 (0.049, 0.413)**	**1.255 (0.238, 2.572)**
Total Tongue	-0.003 (-0.077, 0.036)	-0.021 (-0.443, 0.223)
Epiglottis	0.004 (-0.043, 0.053)	0.023 (-0.281, 0.307)
Fat Pads	0.007 (-0.058, 0.052)	0.046 (-0.355, 0.318)
Pterygoid	-0.031 (-0.123, 0.068)	-0.189 (-0.759, 0.393)
RP Lateral Walls	0.042 (-0.011, 0.121)	0.255 (-0.064, 0.705)
RG Lateral Walls	-0.019 (-0.082, 0.034)	-0.116 (-0.539, 0.190)
Total Lateral Walls	0.009 (-0.061, 0.123)	0.058 (-0.400, 0.649)
Abdominal Fat Volumes		
Abdominal Visceral Fat	-0.043 (-0.183, 0.090)	-0.289 (-1.303, 0.583)
Abdominal Subcutaneous Fat	0.001 (-0.121, 0.119)	0.004 (-0.792, 0.841)
Abdominal Total Fat	-0.018 (-0.153, 0.103)	-0.120 (-1.029, 0.742)

†Estimate of indirect (mediating) effect and bootstrapped 95% confidence interval; ‡All models corrected for age, gender, race and height. Significant values shown in **bold**.

Effect of Weight Loss on Upper Airway Anatomy and the Apnea Hypopnea Index: The Importance of Tongue Fat

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Scientific Knowledge This study examines the effect of weight loss on changes in tongue fat and other upper airway structures in individuals with obesity and sleep apnea assessed with Dixon magnetic resonance imaging. We also examined the influence of changes in upper airway structures with weight loss on improvements in apnea severity. Results indicate that tongue fat is decreased with weight loss and strongly associated with reductions in the apnea-hypopnea index.

What This Study Adds to this Field: This is the first study to show that weight loss decreases tongue fat in patients with sleep apnea, which explains one mechanism for the improvements in sleep apnea with reductions in weight. These findings suggest a potential unique therapeutic target for patients with obesity and sleep apnea, namely, reductions in tongue fat.

DISCLOSURE STATEMENT: The authors of this paper have nothing to declare. Dr. Sarwer has consulting relationships with BARONova, Merz, and NovoNordisk.

This article has an online data supplement.

ABSTRACT

Rationale: Obesity is the primary risk factor for sleep apnea (OSA). Tongue fat is increased in obese persons with OSA, and may explain the relationship between obesity and OSA. Weight loss improves OSA, but the mechanism is unknown.

Objectives: To determine the effect of weight loss on upper airway anatomy in subjects with obesity and OSA. We hypothesized that weight loss would decrease soft tissue volumes and tongue fat and these changes would correlate with reductions in apnea-hypopnea index (AHI).

Methods: Sixty-seven individuals with obesity and OSA (AHI≥10 events/hour) underwent a sleep study and upper airway and abdominal magnetic resonance imaging (MRI) before and after a weight loss intervention (intensive lifestyle modification or bariatric surgery). Airway sizes and soft tissue, tongue fat, and abdominal fat volumes were quantified. Associations between weight loss and changes in these structures, and relationships to AHI changes, were examined.

Measurements and Main Results: Weight loss was significantly associated with reductions in tongue fat, pterygoid and total lateral wall volumes. Reductions in tongue fat were strongly correlated with reductions in AHI (Pearson's rho=0.62, $p<0.0001$); results remained after controlling for weight loss (Pearson's rho=0.36, $p=0.014$). Reduction in tongue fat volume was the primary upper airway mediator of the relationship between weight loss and AHI improvement.

Conclusions: Weight loss reduced volumes of several upper airway soft tissues in subjects with obesity and OSA. Improved AHI with weight loss was mediated by reductions in tongue fat. New treatments that reduce tongue fat should be considered for patients with OSA.

Abbreviations: OSA = obstructive sleep apnea; AHI = apnea/hypopnea index; MRI = magnetic resonance imaging; BMI = body mass index

INTRODUCTION

Over 70% of adults in the United States are overweight (BMI [body mass index] >25 kg/m^2) or obese (BMI >30 kg/m^2) (1). Excess body weight is the primary risk factor for obstructive sleep apnea (OSA) (2-6), and studies support a relationship between weight change and OSA risk or severity (6-8). Weight loss is recommended for the treatment of OSA (9). Across multiple studies, weight losses secondary to a range of dietary and lifestyle modifications are associated with significant reductions in the apnea hypopnea index (AHI) (3, 6-8, 10-15). For example, in the Wisconsin Sleep Cohort, a 1% increase/decrease in body weight was associated with a corresponding 3% increase/decrease in AHI and a 10% weight gain was associated with 6-fold increased risk of developing an AHI >15 events/hour (6). Systematic reviews and meta-analyses of surgical (14) and non-surgical (15) weight loss support this relationship. However, while OSA severity is generally improved, OSA may not be eliminated after weight loss. Why obesity is associated with OSA, why weight loss improves OSA, and why weight gain exacerbates OSA remain unanswered fundamental questions. Addressing these questions is important for optimizing clinical management and personalized OSA treatments.

The mechanisms for improved AHI with weight loss are not well understood. Evidence suggests weight loss may differentially affect AHI in the supine versus non-supine positions (17). Decreased size of the upper airway and larger upper airway soft tissue volumes increase risk for OSA (18). Changes in these anatomical factors with weight loss may explain the improvements in AHI. Our group has shown that tongue fat is increased in obese patients with OSA, compared to obese patients without OSA (19). Thus, reduced tongue fat with weight loss may reduce OSA severity. Similar relationships may exist for other upper airway anatomy implicated in OSA risk (18).

The primary goal of this study was to evaluate changes in upper airway anatomy with weight loss in patients with OSA, and to understand how these changes relate to improvements in AHI. We hypothesized that weight loss would result in corresponding changes in upper airway anatomy (e.g., increased airway size and decreased soft tissue volumes), and these changes, particularly decreased tongue fat, would associate with decreased OSA severity.

METHODS

See details (**Online Supplement)**.

Subjects

This study included subjects with obesity and OSA presenting for bariatric surgery or lifestyle modification intervention for weight loss, recruited from the Penn Center for Sleep Disorders, the University of Pennsylvania's Bariatric Surgery Program, or the Center for Weight and Eating Disorders. The study was approved by the University of Pennsylvania Institutional Review Board. Written informed consent was obtained from all participants. Participants were >18 years-old with an AHI≥10 events/hour on polysomnography (19). Exclusion criteria included inability to undergo magnetic resonance imaging (MRI) or pregnancy. MRI and polysomnography were performed before weight loss treatment and repeated after 6 months.

Weight Loss Protocols

Participants underwent lifestyle modification for weight loss (n=49) or bariatric surgery (n=18; gastric sleeve [n=8], bypass (Roux-en-Y) [n=9] or banding [n=1]). The lifestyle modification was based upon the Diabetes Prevention Program and designed to promote weight loss of 5-10% through caloric restriction, increased physical activity, and behavioral modifications (20).

Polysomnography

Standard polysomnography was conducted as previously described (18, 19).

Magnetic Resonance Imaging

MRI studies were acquired using a 1.5 Tesla MAGNETOM Espree scanner (Siemens Medical Systems, Malvern, PA) as previously described (18, 19, 21, 22). Amira 4.1.2 analysis software (Visage Imaging, San Diego, CA) was utilized to quantify MRI measures in 3 domains: *Airway Sizes* (10 measures) – airway volume, average cross-sectional area, minimum airway area, minimum anterior-posterior distance and minimum lateral distance in the retropalatal (RP) and retroglossal (RG) regions; *Soft Tissue Volumes* (12 measures) – tongue, tongue fat, soft palate, parapharyngeal fat pads, lateral walls, pterygoids, epiglottis, and combined soft tissue volume (the sum of these structures); and *Abdominal Fat Volumes* (3 measures) – total, subcutaneous and visceral fat volumes.

Reproducibility Assessment

Reproducibility of measurements was assessed by calculating intraclass correlation coefficients (ICCs) from data quantified on separate MRIs performed six months apart in a sample of 17 weight stable individuals (defined as follow-up weight within 2.5% of baseline weight). This sample was chosen to represent individuals that did not undergo weight loss intervention. ICCs quantify reproducibility as *poor* (<0.00), *slight* (0.00-0.20), *fair* (0.21-0.40), *moderate* (0.41-0.60), *substantial* (0.61-0.80) and *almost perfect* (0.81-1.00) (23).

Statistical Analysis

Analyses were performed using Stata, Version 14 (StataCorp LP, College Station, TX), SAS Version 9.4 (SAS Institute Inc., Cary, NC) and SPSS 24 (IBM Corp., Armonk, NY). Changes scores were calculated as follow-up minus baseline. Relationships between weight loss and anatomic changes were assessed using unadjusted Pearson's linear correlations and partial

Pearson's correlations adjusted for baseline age, sex, race, height and AHI. Associations between anatomy changes and AHI change were performed similarly. Complementary analyses comparing patients that lost ≥2.5% weight and those with stable/increased weight were conducted using T-tests and linear regression adjusted for age, sex, race, height, AHI and baseline MRI measure (absolute changes only). Within group changes were assessed with paired T-tests. A domain-specific Hochberg step-up correction (24-26) was used to control for multiple comparisons (see **Online Supplement**); p<0.05 was considered nominally significant. Mediators between percent changes in weight and AHI were evaluated using conditional process analysis (27, 28) (**Figure S1**). Bias-corrected 95% confidence intervals (CIs) were estimated via bootstrapping to verify indirect (mediating) effects; mediation was shown if the CI excluded zero (27, 29).

RESULTS

Measurement Reproducibility

To assess measurement reproducibility, we calculated ICCs using data from MRIs taken six months apart in a sample of weight-stable apneic and non-apneic patients (**Table S1**). Nearly all measurements demonstrated substantial (ICC between 0.61-0.80) or almost perfect (ICC between 0.81-1.00) reproducibility. Fat pad volume (ICC=0.353) and both total (ICC=0.489) and subcutaneous (ICC=0.536) abdominal fat volumes showed fair to moderately reproducible.

Participants Characteristics

Sixty-seven patients with OSA were included (**Table 1~~2~~**). Participants were middle-aged (49.4±11.9 years) and obese (BMI of 42.6±8.5 kg/m^2), 40.3% were male and 47.0% were Caucasian. Overall, participants lost 9.5±10.8% of their body weight (p<0.0001) and AHI improved by 30.7±66.7% (p=0.0004). Apneics who lost ≥2.5% weight had a significant AHI reduction (-23.3±21.9; p<0.0001), compared to no change in those that did not (p=0.856) (see **Online Supplement**).

Changes in Anatomy with Weight Loss

To understand how weight loss affected the upper airway and abdominal fat, we assessed Pearson's correlations between percent changes in weight and anatomical structures (**Table 2: A-C~~3~~**). Analyses of absolute changes are presented in **Table S2 (A-C)~~1~~**. Comparisons of patients that lost ≥2.5% weight and those that were weight stable or gained weight are detailed in the Online Supplement (see **Tables S3~~2~~ and S4: A-C~~3~~**).

Airway Sizes

Table 2~~3~~A shows associations between changes in weight and airway sizes. Larger percent decreases in weight were significantly associated with greater percent increases in RG minimum area (Pearson's partial rho=-0.43, p=0.001), controlling for covariates. Significant or nominal correlations were also observed with changes in the shape of the RP airway (**Table 2~~3~~A**). Weight loss was associated with decreased AP distance (partial rho=0.36, p=0.006) and increased lateral distance (partial rho=-0.34, p=0.009) in the RP region. Similar effects on RP airway shape, but not RG minimum area, were observed for absolute changes (**Table S2~~1~~A**). Comparisons between weight loss groups are shown in **Table S3~~2~~A** and **S4~~3~~A** (detailed in the Online Supplement). RP airway sizes and RG minimum area increased in patients that lost weight; changes in RP minimum lateral distance and RG minimum area were different between those that lost weight and those that did not.

Soft Tissue Volumes

Table 2~~3~~B details correlations between percent changes in weight and soft tissue volumes. In adjusted analyses, larger percent reductions in weight were significantly correlated with greater percent reductions in tongue fat (partial rho=0.62, p<0.0001), pterygoid (partial rho=0.40, p=0.002) and total lateral wall (partial rho=0.40, p=0.002) volumes, and nominally correlated with greater percent reductions in RP lateral wall volume (partial rho=0.31, p=0.017). The relationship between reduction in tongue fat volume and percentage change in weight is illustrated in **Figure 1**. Similar results were observed for absolute changes (**Table S2~~1~~B**), with correlations between absolute weight change and change in tongue fat (partial rho=0.48, p=0.001), pterygoid (partial rho=0.37, p=0.005) and total lateral wall (partial rho=0.28, p=0.035) volumes. Most soft tissue measures showed significant decreases among patients with OSA who lost weight (**Online Supplement, Tables S3~~2~~B and S4~~3~~B**), and there were significant differences in tongue fat volume, pterygoid volume and total lateral wall volume changes between weight loss groups. Changes in soft tissue volumes and tongue fat are illustrated in **Figures 2 and 3**.

Abdominal Fat Volumes

Table 2~~3~~C shows relationships between changes in weight and abdominal fat. Strong positive correlations were observed between percent change in weight and percent changes in total (partial rho=0.54, p=0.0001), subcutaneous (partial rho=0.52, p=0.0003) and visceral (partial rho=0.49, p=0.001) abdominal fat in adjusted analyses. Similar associations were seen for absolute changes (**Table S2~~1~~C**). There were larger reductions in each measurement among those who lost weight, compared to no change in those who did not (**Tables S3~~2~~C and S4~~3~~C**). The percentage change in visceral fat was greater than the percentage change in subcutaneous fat among participants who lost weight (p=0.002). Changes in abdominal fat are illustrated in **Figure 4**.

Associations between Changes in Tongue Fat and Abdominal Fat

We assessed correlations between tongue fat and abdominal fat changes, given that both associated with percent changes in weight. Results are detailed in the **Online Supplement**.

Associations between Changes in Anatomy and Changes in AHI

To understand the relationship between changes in upper airway anatomy and OSA severity, we evaluated correlations with percentage changes in AHI (**Tables 34A-C**). Larger percentage reductions in weight were strongly correlated with greater reductions in AHI (partial rho=0.68, p<0.0001). Complementary analyses with absolute changes are presented in **Tables S54 A-C**. Given evidence of positional differences in the AHI response to weight loss (17), analyses examining correlations with changes in supine and non-supine AHI are presented in the **Online Supplement** (**Tables S65 A-C**), among patients with positional AHI≥5 events/hour at baseline. Percentage reductions in weight were strongly correlated with non-supine AHI (partial rho=0.63, p<0.0001), but not supine AHI (partial rho=-0.06, p=0.753).

Airway Sizes

When evaluating the effect of changes in airway size on the AHI (**Table 34A**), controlling for covariates, greater reductions in RP minimum AP distance (partial rho=0.30, p=0.022) and increases in RP minimum lateral distance (partial rho=-0.32, p=0.015) were nominally associated with greater decreases in AHI. Thus, changes in the shape of the RP airway affect OSA severity. In the retroglossal region, larger increases in the minimum area were associated with greater AHI reductions (partial rho=-0.35, p=0.008). These correlations became non-significant after correction for multiple comparisons. There were no significant correlations with absolute changes (**Table S54A**) or positional AHI (**Table S65A**).

Soft Tissue Volumes

Among soft tissue measures (**Table 34B**), greater percentage decreases in tongue fat were associated with larger reductions in AHI (partial rho=0.62, p<0.0001), controlling for clinical covariates. This result remained nominally significant also controlling for weight change (partial rho=0.36, p=0.014), suggesting reduced tongue fat is independently associated with reduced AHI (**Figure 1**). Reductions in RP lateral wall volume were nominally correlated with reductions in AHI (partial rho=0.32, p=0.014); results were not significant controlling for change in weight. Associations between absolute changes in tongue fat and AHI were also observed (**Table S54B**). Percentage reduction in tongue fat was more strongly correlated with reductions in non-supine AHI (partial rho=0.59, p=0.0004) than supine AHI (partial rho=0.22, p=0.260) (**Table S65B**).

Abdominal Fat Volumes

In adjusted analyses, we observed significant correlations between reductions in AHI and reductions in total (partial rho=0.38, p=0.009), subcutaneous (partial rho=0.39, p=0.008) and visceral (partial rho=0.31, p=0.039) abdominal fat (**Table 34C**). Unlike tongue fat, correlations were non-significant controlling for change in weight. Similar results were found for absolute changes (**Table S54C**). Correlations were similar for supine and non-supine AHI, but not significant in the smaller sample (**Table S65C**).

Mediation Analyses

We next evaluated whether changes in specific anatomical structures mediate the relationship between percentage change in weight and percentage change in AHI. In our patients, each 1% change in weight was associated with a corresponding 4% change in AHI (unstandardized total effect [95% CI] = 3.98 [2.74, 5.22]; standardized total effect = 0.648). Percent change in airway size or abdominal fat volumes did not significantly mediate this relationship (**Table 45**). On the other hand, analyses of soft tissue volumes indicated that percent reduction in tongue fat volume was a significant individual mediator between percent change in weight and AHI (unstandardized indirect effect [95% CI] = 1.255 [0.238, 2.572]; standardized indirect effect = 0.225; **Table 45**). Changes in tongue fat accounted for ~30% of the total effect of weight loss on AHI improvement (**Figure 5**). No other soft tissues were significant individual mediators. Thus, these results indicate that change in tongue fat volume is the primary upper airway mediator of the relationship between weight change and change in AHI.

DISCUSSION

This study of patients with obesity and OSA undergoing lifestyle modification or bariatric surgery is the first to show that weight loss decreases tongue fat and the reduction in tongue fat is a mediator of the improvement in AHI. Primary findings include: 1) weight loss was significantly associated with reduced tongue fat volume, pterygoid volume and total lateral wall volume; 2) strong correlations were observed between reductions in tongue fat volume and reductions in AHI; and 3) reduction in tongue fat volume was the primary upper airway mediator of the relationship between reductions in weight and AHI. Beyond providing important mechanistic insights, these results suggest tongue fat could be a potential new target for OSA therapy.

Effect of Weight Loss on Upper Airway Caliber and Surrounding Soft Tissues

Several upper airway measurements changed with weight loss, including retropalatal airway shape and volumes of tongue fat, pterygoid, and the lateral walls. However, other upper airway measurements showed no changes. Thus, weight loss may differentially effect upper airway anatomy; the pathogenesis of this is unclear, but could be genetically determined.

Weight loss reduces adipose tissue volume. Thus, the reduction in tongue fat was expected. However, reductions were also observed for the lateral walls and pterygoid, both of which do not contain fat deposits observable with MRI (although intramyocellular lipid droplets have been observed in the pharyngeal constrictors that make up the lateral walls using electron microscopy) (30). The mechanisms by which weight loss affects soft tissues likely differs for tissues that are primarily fat versus relatively fat-free (31-33). Analyses have shown reduced muscle mass and volume with weight loss (33-37), which could account for reductions in pterygoid and lateral wall volumes. The change in lateral wall volume may be due to other mechanisms, including reduced size of the parapharyngeal fat pads (providing space for the lateral walls) and/or tracheal tug (putting tension and thereby narrowing the lateral walls) secondary to improved lung volume with weight loss.

The repeated trauma of obstruction with OSA may also trigger an edematous response (38). As OSA improves with weight loss, the trauma becomes less severe, reducing the inflammation of pharyngeal tissues. While this would explain the reduction in lateral wall and pterygoid volumes, it does not account for the lack of change in soft palate volume. Since the soft palate contains fat (39), high resolution Dixon imaging (19) may be required to detect fat-specific effects, as seen in the tongue. Alternatively, the soft palate has been shown to be inflamed and fibrosed in apneics (40) and weight loss should not reverse fibrosis. Ultimately, differences in the changes of distinct upper airway soft tissues with weight loss underscores the complexity of these relationships. Understanding the reasons for these differences may provide insight into OSA heterogeneity, and inform personalized treatments.

Tongue Fat as a Potential Therapeutic Target

This study observed strong correlations between tongue fat reduction and improvement in AHI, and mediation analyses supported changes in tongue fat as the primary upper airway mediator between weight loss and AHI reduction. Although the mechanism for this relationship is unknown, reduction in tongue fat affects tongue size and may increase upper airway caliber or improve tongue function. In particular, fat can infiltrate the muscle bundles and affect muscle strength and obesity adversely affects muscle function, with inverse relationships between muscle lipid content and muscle force, velocity, and power (30, 33, 41, 42). Thus, reduced tongue fat should improve muscle function and could prevent collapsibility during sleep. Regardless of mechanism, our results underscore the potential efficacy of OSA therapies that reduce tongue fat.

Although not directly studied, several potential therapies exist. Dixon MRI before and after these interventions is a logical step to determine feasibility and efficacy. Upper airway exercises improve OSA and reduce AHI (43-45); reduced tongue fat is one potential mechanism. Tongue fat may differentially respond to weight loss approaches that vary in dietary composition, although this remains to be investigated. Cold therapies could also potentially remove tongue fat. For example, cryolipolysis is a non-invasive cooling technique that lyses adipocytes and is effective and safe for reducing abdominal and submental fat (46, 47); a similar technique may reduce tongue fat. While experimental, our data provide the foundation for investigation of these therapies through animal or human studies.

Our study may also explain why upper airway surgery is not more effective in treating OSA. Coblation has been used to treat patients with OSA by reducing tongue size, however, it has limited efficacy ~~(~~(48, 49)~~)~~. Coblation does not discriminate between muscle and fat, but instead uses radiofrequency and water to generate a plasma that vaporizes all soft tissue types. Thus, our results demonstrating a specific role for tongue fat volume could explain the observed lack of efficacy. If only fat tissue was removed, coblation could be more effective. Future studies are warranted to study this.

Measurement Reproducibility

Our results confirm reproducibility of MRI measurements at two time points. Nearly all measures showed substantial or almost perfect reproducibility within weight stable individuals. Moderate-to-fair reproducibility was observed for fat pad volume, as well as total and subcutaneous abdominal fat. The likely explanation for this comparatively lower reproducibility is related to lumbar flexion/extension (which is difficult to control) and its effect on the L4-L5 junction, which is the inferior boundary for the abdominal fat measurements. Depending on the amount of lumbar flexion/extension the region of interest analyzed may be different by 1 MRI slice, which can alter the quantitative abdominal fat measures. Nonetheless our results are consistent with our prior studies demonstrating the validity and reproducibility of volumetric measurements quantified by MRI. Previously (21), we demonstrated the accuracy of volumetric measures against a phantom of known volume, the reliability of analyses on the same images, and high reproducibility on repeated MRI taken approximately one month apart. Similarly, our study on tongue fat demonstrated high reproducibility of the measurement technique (19). Moreover, our previous study on abdominal fat measurements demonstrated high reproducibility across multiple raters on repeated images (22). Thus, results from the present study extend evidence of reproducibility to a six month follow-up period in a weight stable population.

Limitations

One limitation of utilizing MRI at multiple timepoints is that anatomic changes may reflect MRI variability (e.g., differences in head and/or neck position), rather than solely the effect of weight loss. The use of standardized protocols and observation that nearly all upper airway measurements showed substantial or almost perfect reproducibility in weight stable patients mitigates this concern. Including medical and surgical weight loss could be viewed as a limitation. However, utilizing multiple types of weight loss interventions increased variability in weight changes (augmenting statistical power) and improves generalizability. Ultimately, we were able to show changes to the upper airway soft tissues and abdominal fat across different treatments and weight loss amounts. However, six months may not be enough time for all structures to show meaningful changes; longer studies should be conducted.

The lack of significant mediation of airway size on the relationship between weight change and AHI may reflect difficulties in measuring airway caliber with MRI during wakefulness. Airway caliber changes during inspiration and expiration (50, 51) but our imaging sequences were performed over several minutes, resulting in average measurements of airway size. Averages may not be sensitive enough to detect mediating effects. In future studies, dynamic MRI should be performed to better capture the effect of awake airway dimensional changes with weight loss.

Conclusions

This study is the first to use volumetric MRI to examine changes in airway sizes, pharyngeal soft tissues and abdominal fat with weight loss in persons with obesity and OSA. We confirmed that our analysis techniques are a reliable means of quantifying the size of upper airway structures over a six month period. Weight loss reduced adipose tissue volumes in the abdomen and upper airway (in particular tongue fat), as well as volumes of soft tissues consisting primarily of fat-free mass (lateral walls, pterygoid). Analyses indicated that reduction in tongue fat was the primary upper airway mediator of the relationship between reductions in weight and AHI. These results elucidate, in part, the mechanism by which weight loss improves OSA and provide targets for potential new therapies in lieu of weight loss.

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Figure Legends

Figure 1. *The relationship of percentage change in tongue fat volume with percentage change in weight and AHI.* The associations between the percentage change in tongue fat and weight loss (left panel) and AHI change (right panel) are illustrated among obese apneics undergoing surgical or medical weight loss. Strong positive correlations were observed between tongue fat change and both measures (Pearson's partial rhos = 0.62, $p<0.0001$), in covariate adjusted analyses. Mediation analyses suggest that percentage change in tongue fat was the primary upper airway mediator between percentage weight loss and percentage reductions in AHI.

Figure 2. *Changes in upper airway soft tissue structures with weight loss.* Three-dimensional reconstructions derived from axial MRI (T1-weighted, spin echo, 3 mm slice thickness), demonstrating changes in selected upper airway soft tissue structures between baseline and 6 month follow-up in a male patient with sleep apnea. Structures include: tongue, defined as the genioglossus muscle (red); soft palate (magenta); parapharyngeal fat pads (yellow); and lateral pharyngeal walls (green). The region of interest extends from the superior appearance of the tongue to the appearance of the hyoid bone.

Figure 3. *Change in tongue fat volume with weight loss.* Three-dimensional reconstruction of tongue (red) and tongue fat (yellow) derived from axial MRI (T1-weighted, spin echo, 3 mm slice thickness) and Dixon fat-only MRI (3 mm slice thickness), demonstrating loss of tongue fat between baseline and a 6 month follow-up visit in the same male apneic as shown in **Figure 2**. The tongue is defined as the genioglossus muscle, and tongue fat is defined as all fat within the genioglossus.

Figure 4. *Change in abdominal fat volumes with weight loss.* Three-dimensional reconstructions of abdomen derived from axial MRI (T1-weighted, spin echo, 10 mm slice thickness) showing fat loss between baseline and a 6 month follow-up visit in the same male apneic as shown in **Figures 2 and 3**. Subcutaneous fat (cyan), visceral fat (yellow), and the liver (red) have been highlighted. Subcutaneous fat is defined as all fat superficial to the abdominal fascia. Visceral fat is defined as all fat within the abdominal fascia that is not part of the spinal column. The region of interest extends from the superior appearance of the liver to the L5-S1 intervertebral disc.

Figure 5. *Mediation of percentage change in weight and AHI by percentage change in tongue fat.* Results of the single mediator model of percent change in tongue fat volume mediating the relationship between percent change in weight and in AHI are shown. Unstandardized path coefficients, interpreted as the expected percentage change in outcome for a 1 percentage change in predictor, of the relationships between percent weight change and percent change in tongue fat (*path a = 1.168****), between percent change in tongue fat and percent change in AHI (*path b = 1.074**), and the remaining direct effect between percent change in weight and percent change in AHI (*path c' = 2.337***) are also shown. Significance of path coefficients is denoted as: *p<0.05, **p<0.01, ***p<0.001.

Table 1: Intraclass Correlation Coefficients for MRI Variables among a weight stable population

Measure	Weight Stable Participants	
	N	ICC‡
RP Airway Volume	17	0.777
RP Cross Sectional Area	17	0.808
RP Minimum Area	17	0.935
RP Minimum AP Distance	17	0.701
RP Minimum Lateral Distance	17	0.889
RG Airway Volume	14	0.844
RG Cross Sectional Area	14	0.856
RG Minimum Area	15	0.849
RG Minimum AP Distance	15	0.746
RG Minimum Lateral Distance	15	0.829
Total Soft Tissue Volume	17	0.944
Soft Palate Volume	17	0.779
Genioglossus Volume	17	0.922
Other Tongue Volume	17	0.721
Tongue Fat Volume	11	0.979
Total Tongue Volume	17	0.946
Epiglottis Volume	17	0.750
Fat Pads Volume	17	0.353
Pterygoid Volume	17	0.694
RP Lateral Walls Volume	17	0.911
RG Lateral Walls Volume	17	0.758
Total Lateral Walls Volume	17	0.910
Abdominal Total Fat Volume	14	0.489
Abdominal Subcutaneous Fat Volume	14	0.536
Abdominal Visceral Fat Volume	14	0.751

‡Intraclass correlation coefficient (ICC) presented as a measure of reproducibility of the indicated measure over time among weight stable individuals; As suggested by Landis and Koch (23), ICC value ranges can be used to qualitatively assess level of agreement as *poor* (<0.00), *slight* (0.00-0.20), *fair* (0.21-0.40), *moderate* (0.41-0.60), *substantial* (0.61-0.80) and *almost perfect* (0.81-1.00). Abbreviations: ICC = intraclass correlation coefficient; RP = retropalatal; RG = retroglossal

Table 12: Demographics of the study sample, overall and by weight loss group

Variable	All Participants	Weight Stable/Gain†	Weight Loss†	p‡
N	67	20	47	–
Age, years	49.4 ± 11.9	51.1 ± 11.8	48.7 ± 12.0	0.472
Male, %	40.3%	35.0%	42.6%	0.564
White, %	47.0%	40.0%	50.0%	0.454
Height, inches	67.1 ± 4.3	66.9 ± 4.6	67.1 ± 4.2	0.814
Weight, pounds				
Baseline	272.0 ± 55.7	287.4 ± 61.7	265.5 ± 52.3	0.143
Follow-up	244.9 ± 54.2	292.9 ± 58.4	224.4 ± 37.1	0.0001
Change	-27.1 ± 33.0§	5.6 ± 11.6§	-41.0 ± 29.1§	<0.0001
% Change	-9.5 ± 10.8§	2.3 ± 4.5§	-14.5 ± 8.5§	<0.0001
BMI, kg/m^2				
Baseline	42.6 ± 8.5	45.3 ± 8.8	41.5 ± 8.2	0.099
Follow-up	38.4 ± 8.5	46.2 ± 8.7	35.1 ± 5.8	<0.0001
Change	-4.2 ± 5.2§	0.9 ± 2.0§	-6.4 ± 4.5§	<0.0001
% Change	-9.5 ± 10.8§	2.3 ± 4.5§	-14.5 ± 8.5§	<0.0001
AHI, events/hour				
Baseline	40.8 ± 28.6	39.2 ± 31.5	41.4 ± 27.6	0.776
Follow-up	25.1 ± 23.6	40.1 ± 24.7	18.6 ± 20.0	0.0004
Change	-16.0 ± 24.5§	0.9 ± 21.9	-23.3 ± 21.9§	0.0001

†Weight loss defined as ≥2.5% decrease in weight from baseline and stable/gain defined as <2.5% decrease in weight; ‡p-value from T-test or chi-squared test comparing values between weight loss and stable/gain groups; ‡within group change significantly different from zero (p<0.05) in paired T-test.

Table 2~~3~~A: Pearson's Correlations between Percent Change in Weight and Percent Change in Airway Dimensions among Patients with OSA

Airway Sizes	Unadjusted			Adjusted		
	N	rho†	p	N	rho‡	p
RP Airway Volume	64	-0.05	0.668	63	-0.09	0.502
RP Cross Sectional Area	64	-0.14	0.285	63	-0.18	0.179
RP Minimum Area	64	0.01	0.942	63	0.01	0.936
RP Minimum AP Distance	64	0.30	0.017	**63**	**0.36**	**0.006**
RP Minimum Lateral Distance	64	-0.26	0.035	63	-0.34	0.009
RG Airway Volume	62	0.08	0.523	61	0.10	0.443
RG Cross Sectional Area	60	0.16	0.224	59	0.14	0.321
RG Minimum Area	61	-0.33	0.008	**60**	**-0.43**	**0.001**
RG Minimum AP Distance	60	0.15	0.253	59	0.12	0.385
RG Minimum Lateral Distance	60	-0.02	0.875	59	-0.05	0.701

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race, AHI and height. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table 2~~3~~B: Pearson's Correlations between Percent Change in Weight and Percent Change in Soft Tissue Volumes among Patients with OSA

Soft Tissue Volumes	Unadjusted			Adjusted		
	N	rho†	p	N	rho‡	p
Combined Soft Tissue	63	0.14	0.268	62	0.18	0.187
Soft Palate	64	-0.15	0.234	63	-0.14	0.303
Genioglossus	64	-0.02	0.866	63	-0.01	0.937
Other Tongue	64	0.01	0.962	63	0.04	0.788
Tongue Fat	**52**	**0.62**	**<0.0001**	**51**	**0.62**	**<0.0001**
Total Tongue	64	-0.03	0.833	63	0.00	0.993
Epiglottis	63	0.07	0.591	62	0.09	0.486
Fat Pads	63	0.08	0.529	62	0.08	0.536
Pterygoid	**64**	**0.42**	**0.001**	**63**	**0.40**	**0.002**
RP Lateral Walls	64	0.25	0.044	63	0.31	0.017
RG Lateral Walls	64	0.19	0.137	63	0.23	0.088
Total Lateral Walls	64	0.32	0.011	**63**	**0.40**	**0.002**

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race, AHI and height. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table 2~~3~~C: Correlations between Percent Change in Weight and Percent Change in Abdominal Fat Measures among Patients with OSA

Abdominal Fat Volume	Unadjusted			Adjusted†		
	N	rho†	p	N	rho‡	p
Abdominal Total Fat	**51**	**0.52**	**0.0001**	**50**	**0.54**	**0.0001**
Abdominal Subcutaneous Fat	**51**	**0.50**	**0.0002**	**50**	**0.52**	**0.0003**
Abdominal Visceral Fat	**51**	**0.46**	**0.001**	**50**	**0.49**	**0.001**

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race, AHI and height. Significant values after Hochberg correction shown in **bold**.

Table 34A: Pearson's correlations between Percent Change in AHI and Percent Change in Airway Size among Patients with OSA

Airway Sizes	Unadjusted			Adjusted Results: Covariates Only			Adjusted Results: Covariates and Weight Change		
	N	rho†	p	N	rho‡	p	N	rho§	p
RP Airway Volume	63	0.13	0.318	62	0.10	0.453	62	0.21	0.124
RP Cross Sectional Area	63	0.07	0.562	62	0.03	0.817	62	0.19	0.155
RP Minimum Area	63	0.02	0.904	62	-0.01	0.946	62	-0.02	0.862
RP Minimum AP Distance	63	0.25	0.045	62	0.30	0.022	62	0.09	0.494
RP Minimum Lateral Distance	63	-0.23	0.076	62	-0.32	0.015	62	-0.14	0.304
RG Airway Volume	61	0.11	0.396	60	0.08	0.558	60	0.03	0.804
RG Cross Sectional Area	59	0.34	0.009	58	0.30	0.025	58	0.28	0.039
RG Minimum Area	60	-0.28	0.033	59	-0.35	0.008	59	-0.11	0.428
RG Minimum AP Distance	59	0.18	0.175	58	0.16	0.233	58	0.09	0.512
RG Minimum Lateral Distance	59	0.10	0.470	58	0.04	0.753	58	0.11	0.428

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table 34B: Pearson's Correlations between Percent Change in AHI and Percent Change in Soft Tissues among Patients with OSA

Soft Tissue Volumes	Unadjusted			Adjusted Results: Covariates Only			Adjusted Results: Covariates and Weight Change		
	N	rho†	p	N	rho‡	p	N	rho§	p
Combined Soft Tissue	62	0.19	0.139	61	0.23	0.090	61	0.17	0.204
Soft Palate	63	-0.16	0.202	62	-0.14	0.305	62	-0.06	0.664
Genioglossus	63	0.13	0.324	62	0.16	0.228	62	0.23	0.082
Other Tongue	63	0.07	0.577	62	0.11	0.401	62	0.13	0.343
Tongue Fat	**51**	**0.61**	**<0.0001**	**50**	**0.62**	**<0.0001**	50	0.36	0.014
Total Tongue	63	0.12	0.340	62	0.18	0.185	62	0.25	0.063
Epiglottis	62	0.05	0.687	61	0.10	0.459	61	0.05	0.712
Fat Pads	62	0.14	0.278	61	0.15	0.281	61	0.12	0.364
Pterygoid	63	0.24	0.055	62	0.19	0.145	62	-0.09	0.488
RP Lateral Walls	63	0.26	0.037	62	0.32	0.014	62	0.18	0.189
RG Lateral Walls	63	0.07	0.600	62	0.04	0.791	62	-0.12	0.357
Total Lateral Walls	63	0.24	0.055	62	0.26	0.051	62	0.03	0.813

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table 34C: Pearson's Correlations between Percent Change in AHI and Percent Change in Abdominal Fat Measures among Patients with OSA

Abdominal Fat Volume	Unadjusted			Adjusted Results					
				Covariates Only			Covariates and Weight Change		
	N	rho†	p	N	rho‡	p	N	rho§	p
Abdominal Total Fat	**50**	**0.37**	**0.008**	**49**	**0.38**	**0.009**	49	-0.04	0.790
Abdominal Subcutaneous Fat	**50**	**0.38**	**0.006**	**49**	**0.39**	**0.008**	49	0.00	0.993
Abdominal Visceral Fat	**50**	**0.29**	**0.041**	**49**	**0.31**	**0.040**	49	-0.11	0.466

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction shown in **bold**.

Table 45: Single mediator modeling results evaluating percent changes in airway, soft tissue and abdominal fat as mediators of the relationship between percent change in weight and AHI

Domain/Measure	Indirect Effect (Bias-Corrected 95% CI)†,‡	
	Standardized	Unstandardized
Airway Sizes		
RP Airway Volume	-0.013 (-0.101, 0.025)	-0.082 (-0.559, 0.157)
RP Average Airway Area Per Slice	-0.025 (-0.116, 0.019)	-0.154 (-0.656, 0.117)
RP Minimum Area	-0.0002 (-0.025, 0.018)	-0.002 (-0.156, 0.107)
RP Minimum AP Distance	0.027 (-0.052, 0.189)	0.166 (-0.311, 1.270)
RP Minimum Lateral Distance	0.037 (-0.033, 0.146)	0.229 (-0.194, 0.913)
RG Airway Volume	0.002 (-0.034, 0.032)	0.013 (-0.205, 0.188)
RG Average Airway Area Per Slice	0.030 (-0.040, 0.120)	0.184 (-0.230, 0.814)
RG Minimum Area	0.040 (-0.060, 0.133)	0.246 (-0.405, 0.667)
RG Minimum AP Distance	0.011 (-0.030, 0.048)	0.065 (-0.180, 0.285)
RG Minimum Lateral Distance	-0.006 (-0.048, 0.023)	-0.034 (-0.296, 0.137)
Soft Tissue Volumes		
Combined Soft Tissues	0.020 (-0.041, 0.057)	0.122 (-0.249, 0.330)
Soft Palate	0.006 (-0.036, 0.039)	0.039 (-0.209, 0.247)
Genioglossus	-0.004 (-0.072, 0.036)	-0.026 (-0.426, 0.235)
Other Tongue	0.002 (-0.053, 0.030)	0.014 (-0.333, 0.170)
Tongue Fat	**0.225 (0.049, 0.413)**	**1.255 (0.238, 2.572)**
Total Tongue	-0.003 (-0.077, 0.036)	-0.021 (-0.443, 0.223)
Epiglottis	0.004 (-0.043, 0.053)	0.023 (-0.281, 0.307)
Fat Pads	0.007 (-0.058, 0.052)	0.046 (-0.355, 0.318)
Pterygoid	-0.031 (-0.123, 0.068)	-0.189 (-0.759, 0.393)
RP Lateral Walls	0.042 (-0.011, 0.121)	0.255 (-0.064, 0.705)
RG Lateral Walls	-0.019 (-0.082, 0.034)	-0.116 (-0.539, 0.190)
Total Lateral Walls	0.009 (-0.061, 0.123)	0.058 (-0.400, 0.649)
Abdominal Fat Volumes		
Abdominal Visceral Fat	-0.043 (-0.183, 0.090)	-0.289 (-1.303, 0.583)
Abdominal Subcutaneous Fat	0.001 (-0.121, 0.119)	0.004 (-0.792, 0.841)
Abdominal Total Fat	-0.018 (-0.153, 0.103)	-0.120 (-1.029, 0.742)

†Estimate of indirect (mediating) effect and bootstrapped 95% confidence interval; ‡All models corrected for age, gender, race and height. Significant values shown in **bold**.

Effect of Weight Loss on Upper Airway Anatomy and the Apnea Hypopnea Index: The Importance of Tongue Fat

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SUPPLEMENTAL METHODS

Subjects

The study used a prospective design of persons with obesity and sleep apnea and who presented for either bariatric surgery or a lifestyle modification intervention for their obesity. Study participants were recruited from the Penn Center for Sleep Disorders outpatient practice and from patients seeking weight-loss treatment at the University of Pennsylvania's Bariatric Surgery Program or a lifestyle modification intervention at the Center for Weight and Eating Disorders. The study was approved by the University of Pennsylvania Institutional Review Board for human studies (protocols 808496 and 809398), and written informed consent was obtained from all participants. All participants were greater than 18 years old and had an apnea-hypopnea index (AHI) ≥10 events/hour as determined by polysomnography which were the criteria used in our previous study examining tongue fat in controls and apneics without weight loss (1). Exclusion criteria included inability to undergo upper airway and abdominal magnetic resonance imaging (MRI) or pregnancy. Participants underwent an MRI and in-laboratory sleep study before beginning weight loss treatment. Participants returned after 6 months for a follow-up MRI and in-laboratory sleep study.

Medical and Surgical Weight Loss Protocols

Participants undergoing a lifestyle modification program for weight loss (n=49) or bariatric surgery (n=18) were invited to participate. Patients undergoing bariatric surgery had gastric sleeve procedure (n=8), gastric bypass (Roux-en-Y) (n=9) or gastric banding (n=1). The lifestyle modification program was based upon the Diabetes Prevention Program and designed to promote a weight loss of 5-10% of initial body weight through caloric restriction, increased physical activity, and behavioral modification strategies (2). The calorie goals were 1200-1500 kcal/day for those who weighed <250 pounds, and 1500-1800 kcal/day for those whose weight exceeded 250 pounds. Dietary composition was aligned with that recommended by the NHLBI guidelines on the treatment of obesity (2) and included recommendations to consume <7% saturated fat, <10% polyunsaturated fat, <20% monounsaturated fats, 25-30% total fat, 50-60% carbohydrates, and 15% protein. Participants were prescribed a program of physical activity consisting of walking 4 times per week, starting at 10 minutes per session and building to 30 minutes per session over a 12-week period. Participants had 30-minute individual sessions with a registered dietitian with extensive experience in behavioral weight control counseling. Each patient received 24 weekly individual counseling sessions during the 6-month treatment period. Participants were instructed on how to comply with the dietary prescription and in traditional behavioral methods of weight control such as self-monitoring, stimulus control, slow eating, and related behaviors.

Magnetic Resonance Imaging (MRI)

Upper airway and abdominal MRI studies were acquired using a 1.5 Tesla MAGNETOM Espree scanner (Siemens Medical Systems, Malvern, PA), as described in our previous publications (1, 3-5). Three different imaging sequences were utilized as reported in our previous publications: 1) T1-weighted spin echo imaging (for airway and surrounding upper airway soft tissue structures) (3, 5); 2) Dixon imaging for tongue fat measurements (1); and 3) T1-weighted gradient recalled echo imaging for abdominal fat imaging (4).

Analysis of the Upper Airway, Surrounding Soft Tissue Structures and Abdominal Fat

The MRI analysis was performed twice (baseline and after 6 months) and split into 3 different domains: 1) *upper airway analysis* (airway volume, average cross-sectional area, minimum airway area, minimum anterior-posterior distance and minimum lateral distance) in the retropalatal (RP) and retroglossal (RG) regions; 2) *volumetric analysis of the upper airway soft tissues structures* (tongue, tongue fat, soft palate, parapharyngeal fat pads, lateral walls, pterygoids, epiglottis, and combined soft tissue volume [equal to the sum of these structures]); and 3) *abdominal fat volumes* (total, subcutaneous and visceral). As previously described, Amira 4.1.2 image analysis software (Visage Imaging, San Diego, CA), was utilized to quantify the upper airway, the surrounding soft tissue structures(1, 5, 6), tongue fat (1) and abdominal subcutaneous/visceral fat (4). Amira is a software program that allows the technician to segment the upper airway soft tissues structures using thresholding based on grayscale of the specific tissue (tongue, pharyngeal lateral walls, parapharyngeal fat pads, etc). Amira requires the technician to outline the pharyngeal structures. The technician was blinded to the pre-post-weight loss status of the patients and performed the analysis in the same manner in each subject. We have shown that our upper airway soft tissue and tongue fat analysis measurements are reproducible and accurate(1, 5). Accuracy of our fat volume estimates have been assessed previously (1) by performing Dixon MRI of a hamburger and steak before and after injection of a known volume of fat (lard) into the tissue (6 cc added to the steak and 3 cc added to the hamburger) and comparing the results to the known quantity.

Although fat-weighted Dixon images provide a reproducible objective measure adipose tissue, the boundaries of soft tissues with Dixon imaging are not as distinct in comparison to the standard spin-echo images (1). Therefore the boundaries of the tongue on each MR axial slice was determined on standard axial T1 spin-echo MR images, and then superimposed onto the axial Dixon fat-only MR images in order to select all fat within that region.. The grayscale setting to segment tongue fat was chosen based on the grayscale intensity of the surrounding subcutaneous and neck fat allowing us to standardize the tongue fat intensity across all subjects (1). We have shown that this analysis technique for tongue fat is highly reproducible (1). The technician performing these MRI analyses was blinded to polysomnography results and the pre-post weight loss state of each subject.

Reproducibility Assessment

To understand the reproducibility of MRI analyses, we compared measurements quantified from MRIs performed 6 months apart in an available sample of 17 individuals (n=13 OSA, 4 non-OSA) that were weight stable over the follow-up period (defined as follow-up weight within 2.5% of baseline weight). Using these repeated measurements, we calculated the intraclass correlation coefficient (ICC) for each of our anatomy measures of interest. As suggested by Landis and Koch (7), ICC value ranges can be used to qualitatively assess level of reproducibility as *poor* (<0.00), *slight* (0.00-0.20), *fair* (0.21-0.40), *moderate* (0.41-0.60), *substantial* (0.61-0.80) and *almost perfect* (0.81-1.00).

Statistical Analysis

Data are summarized using means and standard deviations (continuous) or frequencies and percentages (categorical). To summarize changes over the follow-up period, we calculated subject-specific percentage and absolute change scores as follow-up minus baseline values. Primary analyses examining the relationship between weight loss and anatomic changes were performed using Pearson's correlations, unadjusted and controlling for relevant baseline covariates (age, sex, race, height and AHI). Associations between change in upper airway anatomy (or weight) and change in AHI were performed in a similar manner. These analyses were performed unadjusted, controlling for clinical covariates, and further adjusted for percentage change in weight to assess whether individual structures were associated with changes in AHI independent of change in weight. As a complementary analysis, we also quantified and compared change scores in patients that lost at least 2.5% weight and those that were stable or gained weight. Comparisons between groups were performed using T-tests or linear regression models with and without covariate adjustment. In addition to the above covariates, baseline values of the variable of interest were included in models comparing absolute change between groups. Significance of within group changes were assessed with paired T-tests. Where presented, statistical comparisons of correlation coefficients were performed by deriving a non-parametric p-value based on the observed distribution of differences in correlations from 1,000 bootstrapped samples (8, 9).

To test whether associations between percent weight change and percent AHI improvements are mediated by specific upper airway or soft tissue anatomy percent changes, we utilized mediation modeling (10). Mediation analyses were conducted by PROCESS, a conditional process modeling program that utilizes an ordinary least squares path analytical framework to test for both direct and indirect effects (11). To comprehensively examine upper airway mediators, we first tested all putative mediators individually using single mediator models. Based on the results of these single mediator models, we then created a combined, parallel multiple mediator model including any significant individual mediators to examine the most influential mediators in the presence of all possible mediators (see **Figure S1** for hypothesized mediation models). Bias-corrected 95% confidence intervals (CIs) were estimated using bootstrapping (n=5000 samples) to verify indirect (mediating) effects; estimates are presented unstandardized and standardized (to allow direct comparison of indirect effects across proposed mediators). The indirect effect was considered significant and mediation demonstrated if this confidence interval did not contain zero. (10, 12). This approach computes more accurate confidence intervals of indirect effects than other commonly used methods (10) and provides higher power while maintaining control over the Type I error rate (13). Baseline age, sex, race, and height were controlled for all mediation models.

We utilized a domain-specific Hochberg correction (14-16) to control for multiple comparisons when determining statistical significance of associations with individual upper airway anatomical measures. As described by Hochberg and Benjamini (15), for a given set of *m* hypotheses:

> *This procedure starts by examining the largest p-value $P_{(m)}$. If $P_{(m)} \leq \alpha$, then $H_{(m)}$ and all other hypotheses are rejected. If not, $H_{(m)}$ is not rejected and one proceeds to compare $P_{(m-1)}$ with $\alpha/2$. If the former is smaller, then $H_{(m-1)}$ and all hypotheses with smaller p-values are rejected. Generally, one proceeds from highest to lower p-values, retaining $H_{(i)}$ if its p-value satisfies $P_{(i)}$*

$> \alpha/(m - i + 1)$. One stops the procedure at the first ordered hypothesis when that inequality is reversed. This hypothesis is rejected and so are all hypotheses with lower or equal p-values.

In addition to this approach for determining statistically significant results, any unadjusted $p<0.05$ was considered nominally significant evidence. Significance in multivariate models was assessed using the confidence interval approach, detailed above. Analyses were conducted using Stata, Version 14 (StataCorp LP, College Station, TX), SAS Software Version 9.4 (SAS Institute Inc., Cary, NC) and SPSS 24 (IBM Corp., Armonk, NY).

Our sample size of 67 apneics provided adequate power (80%) for detecting correlations of 0.34 at a nominal level of significance ($p<0.05$). Similarly, in analyses comparing apneics who lost weight (n=47) to those who were weight stable or gained weight (n=20), we had at least 80% power to detect standardized differences of 0.76. These correspond to moderate or large effects as defined by Cohen (17). Thus, the study was powered for meaningful effect sizes. Non-significant associations for smaller effect sizes should be interpreted with some caution, as it is possible that these represent real associations that the current sample is underpowered to declare significant (e.g., false negatives).

SUPPLEMENTAL RESULTS

Patient characteristics of weight loss groups

Demographic characteristics of the sample are shown in **Table 1**. Forty-seven (70.1%) patients lost ≥2.5% body weight (average change -14.5±8.5% [p<0.0001]), compared to 20 (29.9%) that were weight stable or gained weight (average change 2.3±4.5% [p=0.036]). Patients who lost weight had a significant AHI reduction (-23.3±21.9; p<0.0001), compared to no change in those that did not (p=0.856).

Comparison of weight loss interventions

All participant in the bariatric surgery arm (n=18) lost at least 2.5% body weight, while in those undergoing lifestyle modification a total of 29 (59.2%) lost at least 2.5% body weight and 20 (40.8%) were weight stable or gained weight. Among participants that lost weight, those that underwent bariatric surgery had greater weight loss than those that underwent intensive lifestyle modification (-19.2±6.8% vs. -11.6±8.2%, p=0.002). Relatedly, there was greater improvement in AHI among patients who lost weight with bariatric surgery than those who lost weight with intensive lifestyle modification (-73.8±17.8% vs. -49.6±36.5%, p=0.004).

Changes in Upper Airway Anatomy in Weight Loss and Weight Stable/Gain Patients

Airway Sizes

Secondary comparisons of percent changes in airway size between weight loss and weight stable/gain patients are shown in **Table S3A**. Patients who lost ≥2.5% weight showed increased retropalatal airway volume (p=0.007), cross-sectional area (p=0.001), minimum area (p=0.003) and minimum lateral distance (p=0.001), as well as a large increase in retroglossal minimum area (p=0.001). After covariate adjustment, differences in the percentage changes in the RP minimum lateral distance (p=0.020) and RG minimum area (p=0.023) were nominally different between weight loss groups

(**Table S3A**). Similar results were observed when comparing absolute changes (**Table S4A**), although the increased RG minimum area was nominal (p=0.032) among the weight loss group. There was also a significant absolute decrease in RP minimum AP distance (p=0.003) among the weight loss group. After adjustment, differences in RP minimum AP (p=0.022) and lateral (p=0.033) distance remained nominally different between groups.

Soft Tissue Volumes

Supporting results observed in correlational analysis, most soft tissue measures showed significant decreases in volume among patients with OSA who lost weight (**Table S3B**), including combined soft tissue (p<0.0001), genioglossus (p=0.001), tongue fat (p<0.0001), total tongue (p=0.009), fat pads (p<0.0001), pterygoid (p<0.0001), RP lateral walls (p<0.0001) and total lateral walls (p<0.0001). Compared to patients without weight loss, there were significant differences in the change in tongue fat volume (p<0.0001), pterygoid volume (p=0.001) and total lateral wall volume (p=0.001). Differences remained significant controlling for covariates (**Table S3B**). Similar results were observed for absolute changes (**Tables S4B**); in adjusted analyses, we also observed a significant difference in the absolute change in fat pad volume between groups (p=0.004).

Abdominal Fat Volumes

Among patients who lost ≥2.5% weight, there were large percentage reductions in each measurement (**Table S3C**), compared to no significant changes in those who did not lose weight. Differences between groups were statistically significant in adjusted analyses. Similar results were seen for absolute changes (**Table S4C**), with significant differences between groups in adjusted analysis.

Associations between Changes in Tongue Fat and Abdominal Fat

Given correlations between percent change in weight and percent changes in tongue and abdominal fat volumes, we assessed the correlations between tongue and abdominal fat changes. In covariate adjusted analyses, percentage change in tongue fat was positively associated with percentage changes in total (partial rho = 0.48, p=0.005), subcutaneous (partial rho = 0.40, p=0.021), and visceral (partial rho = 0.55, p=0.0009) fat volumes. Correlations between the percentage changes in tongue fat and both total abdominal fat (p=0.034) and subcutaneous abdominal fat (p=0.026), but not visceral abdominal fat (p=0.154), were smaller than the correlation between percentage changes in tongue fat and weight. Thus, there is some evidence of a stronger relationship of percent changes in tongue fat with changes in weight.

Figure S1. *Illustration of hypothesized single and multiple mediation models.* The hypothesized mediation models examined through conditional process analysis are illustrated. In particular, the direct effect of percent change in weight on percentage change in AHI (*path c*) is shown in the top schematic. The middle schematic shows the evaluated single mediator model of mediation by an individual upper airway variable, including the relationship between weight percent change and anatomy (*path a*), between anatomy and percentage change in AHI (*path b*) and the remaining direct effect of weight percent change on AHI percent change (*path c'*). Finally, the single mediator model is extended to show the hypothesized multiple mediator model, including N single mediators with specific individual a_N and b_N path effects.

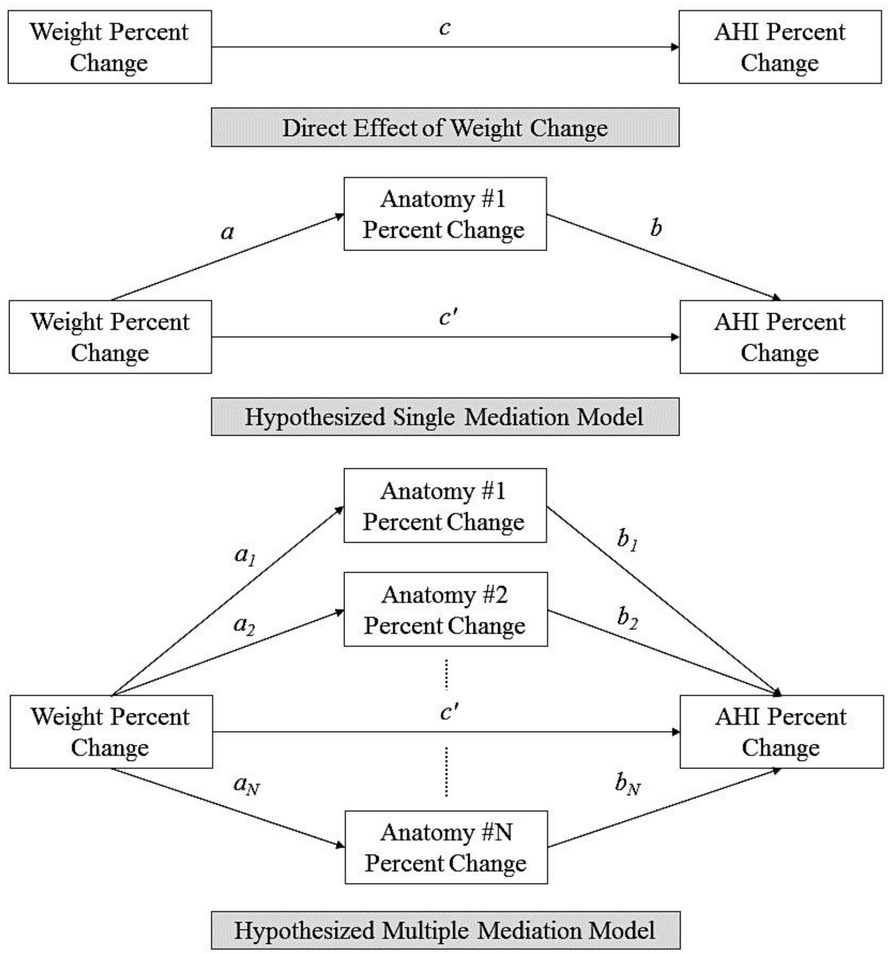

Table S1: Intraclass Correlation Coefficients for MRI Variables Among a Weight Stable Population

Measure	Weight Stable Participants	
	N	ICC†
RP Airway Volume	17	0.777
RP Cross Sectional Area	17	0.808
RP Minimum Area	17	0.935
RP Minimum AP Distance	17	0.701
RP Minimum Lateral Distance	17	0.889
RG Airway Volume	14	0.844
RG Cross Sectional Area	14	0.856
RG Minimum Area	15	0.849
RG Minimum AP Distance	15	0.746
RG Minimum Lateral Distance	15	0.829
Total Soft Tissue Volume	17	0.944
Soft Palate Volume	17	0.779
Genioglossus Volume	17	0.922
Other Tongue Volume	17	0.721
Tongue Fat Volume	11	0.979
Total Tongue Volume	17	0.946
Epiglottis Volume	17	0.750
Fat Pads Volume	17	0.353
Pterygoid Volume	17	0.694
RP Lateral Walls Volume	17	0.911
RG Lateral Walls Volume	17	0.758
Total Lateral Walls Volume	17	0.910
Abdominal Total Fat Volume	14	0.489
Abdominal Subcutaneous Fat Volume	14	0.536
Abdominal Visceral Fat Volume	14	0.751

†Intraclass correlation coefficient (ICC) presented as a measure of reproducibility of the indicated measure over time among weight stable individuals; As suggested by Landis and Koch (23), ICC value ranges can be used to qualitatively assess level of agreement as *poor* (<0.00), *slight* (0.00-0.20), *fair* (0.21-0.40), *moderate* (0.41-0.60), *substantial* (0.61-0.80) and *almost perfect* (0.81-1.00). Abbreviations: ICC = intraclass correlation coefficient; RP = retropalatal; RG = retroglossal

Table S2A: Pearson's Correlations between Absolute Change in Weight and Absolute Change in Airway Size among Patients with OSA

Airway Sizes	Unadjusted			Adjusted		
	N	rho†	p	N	rho‡	p
RP Airway Volume	64	-0.16	0.211	63	-0.21	0.110
RP Cross Sectional Area	64	-0.21	0.103	63	-0.27	0.037
RP Minimum Area	64	-0.03	0.789	63	-0.05	0.688
RP Minimum AP Distance	**64**	**0.36**	**0.003**	**63**	**0.49**	**0.0001**
RP Minimum Lateral Distance	64	-0.33	0.008	**63**	**-0.40**	**0.002**
RG Airway Volume	62	0.08	0.535	61	0.11	0.434
RG Cross Sectional Area	60	0.06	0.662	59	0.04	0.761
RG Minimum Area	61	-0.01	0.934	60	-0.13	0.335
RG Minimum AP Distance	60	0.26	0.046	59	0.24	0.078
RG Minimum Lateral Distance	60	-0.03	0.842	59	-0.04	0.757

†Unadjusted Pearson's linear correlations; ‡Partial Pearson's correlation adjusted for age, gender, race, AHI and height. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S2B: Pearson's Correlations between Absolute Change in Weight and Absolute Change in Soft Tissue Volumes among Patients with OSA

Soft Tissue Volumes	Unadjusted			Adjusted		
	N	rho†	p	N	rho‡	p
Combined Soft Tissue	63	0.09	0.488	62	0.10	0.456
Soft Palate	64	-0.12	0.338	63	-0.12	0.363
Genioglossus	64	-0.03	0.825	63	-0.03	0.799
Other Tongue	64	-0.06	0.649	63	-0.03	0.811
Tongue Fat	**52**	**0.47**	**0.0004**	**51**	**0.48**	**0.001**
Total Tongue	64	-0.06	0.639	63	-0.05	0.727
Epiglottis	63	0.04	0.737	62	0.06	0.632
Fat Pads	63	0.06	0.631	62	0.04	0.787
Pterygoid	**64**	**0.37**	**0.002**	63	0.37	0.005
RP Lateral Walls	64	0.16	0.212	63	0.15	0.267
RG Lateral Walls	64	0.17	0.171	63	0.22	0.094
Total Lateral Walls	64	0.24	0.054	63	0.28	0.035

†Unadjusted Pearson's linear correlations; ‡Partial Pearson's correlation adjusted for age, gender, race, AHI and height. Significant values represented in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S2C: Pearson's Correlations between Absolute Change in Weight and Absolute Change in Abdominal Fat Measures among Patients with OSA

Abdominal Fat Volume	Unadjusted			Adjusted		
	N	rho†	p	N	rho‡	p
Abdominal Total Fat	**51**	**0.57**	**<0.0001**	**50**	**0.58**	**<0.0001**
Abdominal Subcutaneous Fat	**51**	**0.56**	**<0.0001**	**50**	**0.57**	**<0.0001**
Abdominal Visceral Fat	**51**	**0.49**	**0.0003**	**50**	**0.54**	**0.0001**

†Unadjusted Pearson's linear correlations; ‡Partial Pearson's correlation adjusted for age, gender, race, AHI and height. Significant values represented in **bold**.

Table S3A: Percent Changes in Airway Size in Patients with OSA based on weight change

Airway Sizes	Weight Stable/Gain			Weight Loss			p_{unadj}‡	p_{adj}§
	N	Mean ± SD	p†	N	Mean ± SD	p†		
RP Airway Volume	19	13.8 ± 47.0	0.216	**45**	**21.5 ± 51.3**	**0.007**	0.579	0.512
RP Cross Sectional Area	19	15.4 ± 42.0	0.127	**45**	**24.4 ± 47.5**	**0.001**	0.475	0.374
RP Minimum Area	19	17.8 ± 58.1	0.197	**45**	**55.9 ± 116.7**	**0.003**	0.088	0.158
RP Minimum AP Distance	19	9.7 ± 42.6	0.332	45	-3.1 ± 77.3	0.789	0.399	0.426
RP Minimum Lateral Distance	19	1.1 ± 30.6	0.879	**45**	**33.8 ± 65.6**	**0.001**	0.009	0.020
RG Airway Volume	17	19.6 ± 29.6	0.015	45	7.8 ± 35.7	0.149	0.230	0.317
RG Cross Sectional Area	17	19.6 ± 36.4	0.042	43	6.3 ± 28.7	0.160	0.140	0.197
RG Minimum Area	17	21.7 ± 57.1	0.136	**44**	**424.6 ± 778.8**	**0.001**	**0.001**	0.023
RG Minimum AP Distance	17	8.1 ± 40.4	0.421	43	7.0 ± 48.2	0.343	0.938	0.998
RG Minimum Lateral Distance	17	13.3 ± 44.3	0.233	43	16.3 ± 54.4	0.055	0.840	0.668

†p-value from paired T-test examining significance of within group change; ‡p-value from T-test comparing changes between weight loss and weight stable/gain; §p-value adjusted for age, sex, race, AHI and height. Significant values after Hochberg correction represented in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S3B: Percent Changes in Soft Tissue Volumes in Patients with OSA based on weight change

Soft Tissue Volumes	Weight Stable/Gain			Weight Loss			p_{unadj}‡	p_{adj}§
	N	Mean ± SD	p†	N	Mean ± SD	p†		
Combined Soft Tissue	19	-3.3 ± 6.3	0.035	**44**	**-7.4 ± 9.0**	**<0.0001**	0.077	0.057
Soft Palate	19	-8.6 ± 22.7	0.115	45	-0.1 ± 24.0	0.986	0.190	0.197
Genioglossus	19	-3.0 ± 7.3	0.092	**45**	**-5.1 ± 10.0**	**0.001**	0.409	0.318
Other Tongue	19	-2.4 ± 25.0	0.682	45	-0.8 ± 28.4	0.859	0.828	0.951
Tongue Fat	13	6.8 ± 11.5	0.055	**39**	**-19.9 ± 17.8**	**<0.0001**	**<0.0001**	**<0.0001**
Total Tongue	19	-3.7 ± 6.5	0.023	**45**	**-4.6 ± 11.4**	**0.009**	0.692	0.577
Epiglottis	19	14.7 ± 38.2	0.111	44	3.6 ± 40.1	0.557	0.309	0.211
Fat Pads	19	-15.9 ± 26.0	0.016	**44**	**-28.3 ± 22.2**	**<0.0001**	0.058	0.078
Pterygoid	19	1.5 ± 16.9	0.704	**45**	**-12.0 ± 13.4**	**<0.0001**	**0.001**	**0.004**
RP Lateral Walls	19	-2.6 ± 16.4	0.504	**45**	**-11.9 ± 15.1**	**<0.0001**	0.032	0.022
RG Lateral Walls	19	14.3 ± 22.0	0.011	45	-2.8 ± 24.2	0.441	0.010	0.020
Total Lateral Walls	19	3.4 ± 14.8	0.333	**45**	**-9.3 ± 11.8**	**<0.0001**	**0.001**	**0.001**

†p-value from paired T-test examining significance of within group change; ‡p-value from T-test comparing changes between weight loss and weight stable/gain; §p-value adjusted for age, sex, race, AHI and height. Significant values after Hochberg correction represented in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S3C: Percent Changes in Abdominal Fat Volumes in Patients with OSA based on weight change

Abdominal Fat Volume	Weight Stable/Gain			Weight Loss			p_{unadj}‡	p_{adj}§
	N	Mean ± SD	p†	N	Mean ± SD	p†		
Abdominal Total Fat	15	-0.1 ± 38.4	0.991	**36**	**-21.1 ± 17.8**	**<0.0001**	0.059	**0.013**
Abdominal Subcutaneous Fat	15	-1.4 ± 36.0	0.883	**36**	**-19.4 ± 18.2**	**<0.0001**	0.084	**0.024**
Abdominal Visceral Fat	15	9.5 ± 64.4	0.576	**36**	**-25.9 ± 21.6**	**<0.0001**	0.055	**0.007**

†p-value from paired T-test examining significance of within group change; ‡p-value from T-test comparing changes between weight loss and weight stable/gain; §p-value adjusted for age, sex, race, AHI and height. Significant values after Hochberg correction represented in **bold.**

Table S4A: Absolute Changes in Airway Size in Patients with OSA based on weight change

Airway Sizes	Weight Stable/Gain			Weight Loss			p_{unadj}‡	p_{adj}§
	N	Mean ± SD	p†	N	Mean ± SD	p†		
RP Airway Volume, mm^3	19	103.8 ± 1773.5	0.802	45	377.2 ± 1434.5	0.085	0.519	0.754
RP Cross Sectional Area, mm^2	19	32.0 ± 134.3	0.312	45	53.1 ± 125.6	0.007	0.551	0.632
RP Minimum Area, mm^2	19	3.3 ± 23.1	0.547	45	13.4 ± 39.1	0.027	0.205	0.232
RP Minimum AP Distance, mm	19	0.47 ± 3.23	0.533	**45**	**-3.54 ± 7.52**	**0.003**	**0.004**	0.022
RP Minimum Lateral Distance, mm	19	-0.26 ± 2.42	0.642	**45**	**2.24 ± 4.79**	**0.003**	0.008	0.033
RG Airway Volume, mm^3	17	1030.8 ± 1823.3	0.033	45	-25.9 ± 2395.0	0.943	0.105	0.286
RG Cross Sectional Area, mm^2	17	88.7 ± 242.7	0.151	43	18.5 ± 195.6	0.539	0.247	0.379
RG Minimum Area, mm^2	17	16.9 ± 81.3	0.404	44	39.6 ± 118.6	0.032	0.471	0.848
RG Minimum AP Distance, mm	17	0.26 ± 4.42	0.813	43	-0.52 ± 4.49	0.456	0.548	0.220
RG Minimum Lateral Distance, mm	17	0.23 ± 5.57	0.866	43	0.72 ± 6.10	0.443	0.776	0.944

†p-value from paired T-test examining significance of within group change; ‡p-value from T-test comparing changes between weight loss and weight stable/gain; §p-value adjusted for age, sex, race, AHI, height and baseline airway size. Significant values after Hochberg correction represented in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S4B: Absolute Changes in Soft Tissue Volumes in Patients with OSA based on weight change

Soft Tissue Volumes (mm^3)	Weight Stable/Gain			Weight Loss			p_{unadj}‡	p_{adj}§
	N	Mean ± SD	p†	N	Mean ± SD	p†		
Combined Soft Tissue	19	-6959.9 ± 11999.6	0.021	**44**	**-15100.3 ± 19816.8**	**<0.0001**	0.050	0.054
Soft Palate	19	-894.0 ± 1866.5	0.051	45	-191.4 ± 1746.8	0.466	0.155	0.381
Genioglossus	19	-3014.8 ± 7137.1	0.082	**45**	**-5284.8 ± 10333.8**	**0.001**	0.387	0.306
Other Tongue	19	-2286.4 ± 9895.2	0.327	45	-1197.6 ± 10204.6	0.435	0.695	0.444
Tongue Fat	13	1864.4 ± 3173.6	0.056	**39**	**-5536.7 ± 5110.1**	**<0.0001**	**<0.0001**	**0.0001**
Total Tongue	19	-5301.2 ± 8809.8	0.017	**45**	**-6482.4 ± 16082.5**	**0.010**	0.708	0.512
Epiglottis	19	124.3 ± 544.3	0.333	44	-33.5 ± 613.7	0.719	0.337	0.028
Fat Pads	19	-1504.5 ± 2276.3	0.010	**44**	**-2219.8 ± 1927.1**	**<0.0001**	0.206	**0.004**
Pterygoid	19	232.8 ± 4043.8	0.805	**45**	**-3149.4 ± 3444.5**	**<0.0001**	**0.001**	**0.0002**
RP Lateral Walls	19	-684.4 ± 2108.1	0.174	**45**	**-1795.2 ± 2381.8**	**<0.0001**	0.083	0.016
RG Lateral Walls	19	1067.2 ± 2065.4	0.037	45	-807.4 ± 3044.4	0.082	0.017	0.040
Total Lateral Walls	19	382.8 ± 2808.7	0.560	**45**	**-2602.7 ± 3566.0**	**<0.0001**	**0.002**	**0.001**

†p-value from paired T-test examining significance of within group change; ‡p-value from T-test comparing changes between weight loss and weight stable/gain; §p-value adjusted for age, sex, race, AHI, height and baseline soft tissue volume. Significant values after Hochberg correction represented in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S4C: Absolute Changes in Abdominal Fat Volumes in Patients with OSA based on weight change

Abdominal Fat Volume (mm^3)	Weight Stable/Gain			Weight Loss			p_{unadj}‡	p_{adj}§
	N	Mean ± SD	p†	N	Mean ± SD	p†		
Abdominal Total Fat	15	-1337194 ± 6041271	0.406	**36**	**-3825576 ± 4108429**	**<0.0001**	0.094	**0.002**
Abdominal Subcutaneous Fat	15	-1019828 ± 4808678	0.425	**36**	**-2898336 ± 3418169**	**<0.0001**	0.120	**0.006**
Abdominal Visceral Fat	15	-317367 ± 1441512	0.408	**36**	**-927240 ± 958480**	**<0.0001**	0.082	**0.001**

†p-value from paired T-test examining significance of within group change; ‡p-value from T-test comparing changes between weight loss and weight stable/gain; §p-value adjusted for age, sex, race, AHI, height and baseline abdominal fat volume. Significant values after Hochberg correction represented in **bold.**

Table S5A: Correlations between Absolute Change in AHI and Absolute Change in Airway Size among Patients with OSA

Airway Sizes	Unadjusted			Adjusted Results: Covariates Only†			Adjusted Results: Covariates and Weight Change‡		
	N	rho†	p	N	rho‡	p	N	rho§	p
RP Airway Volume	63	0.15	0.241	62	0.08	0.576	62	0.24	0.077
RP Cross Sectional Area	63	0.12	0.347	62	0.05	0.737	62	0.24	0.067
RP Minimum Area	63	-0.04	0.726	62	-0.05	0.712	62	-0.03	0.833
RP Minimum AP Distance	63	0.26	0.038	62	0.30	0.022	62	0.04	0.762
RP Minimum Lateral Distance	63	-0.17	0.173	62	-0.18	0.183	62	0.06	0.681
RG Airway Volume	61	-0.12	0.371	60	-0.14	0.292	60	-0.23	0.090
RG Cross Sectional Area	59	0.02	0.867	58	0.00	0.973	58	-0.02	0.873
RG Minimum Area	60	-0.06	0.657	59	-0.07	0.601	59	0.00	0.977
RG Minimum AP Distance	59	0.12	0.347	58	0.16	0.249	58	0.01	0.944
RG Minimum Lateral Distance	59	-0.10	0.429	58	-0.23	0.102	58	-0.22	0.107

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction represented in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S5B: Correlations between Absolute Change in AHI and Absolute Change in Soft Tissues among Patients with OSA

Soft Tissue Volumes	Unadjusted			Adjusted Results: Covariates Only†			Adjusted Results: Covariates and Weight Change‡		
	N	rho†	p	N	rho‡	p	N	rho§	p
Combined Soft Tissue	62	0.09	0.482	61	0.05	0.698	61	0.01	0.913
Soft Palate	63	-0.06	0.625	62	-0.08	0.554	62	-0.01	0.944
Genioglossus	63	0.03	0.794	62	0.05	0.735	62	0.09	0.507
Other Tongue	63	-0.04	0.734	62	-0.07	0.618	62	-0.05	0.721
Tongue Fat	**51**	**0.47**	**0.001**	**50**	**0.47**	**0.001**	50	0.29	0.057
Total Tongue	63	-0.01	0.947	62	-0.02	0.901	62	0.03	0.846
Epiglottis	62	0.13	0.316	61	0.08	0.539	61	0.06	0.673
Fat Pads	62	0.09	0.501	61	0.09	0.483	61	0.09	0.505
Pterygoid	63	0.28	0.024	62	0.19	0.148	62	-0.02	0.903
RP Lateral Walls	63	0.30	0.018	62	0.25	0.061	62	0.21	0.120
RG Lateral Walls	63	-0.05	0.697	62	-0.10	0.448	62	-0.25	0.058
Total Lateral Walls	63	0.15	0.233	62	0.07	0.594	62	-0.08	0.574

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction represented in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S5C: Correlations between Absolute Change in AHI and Absolute Change in Abdominal Fat Measures among Patients with OSA

Abdominal Fat Volume	Unadjusted			Adjusted Results					
				Covariates Only			Covariates and Weight Change		
	N	rho†	p	N	rho‡	p	N	rho§	p
Abdominal Total Fat	50	0.31	0.029	**49**	**0.34**	**0.021**	49	0.03	0.829
Abdominal Subcutaneous Fat	50	0.28	0.051	**49**	**0.33**	**0.027**	49	0.02	0.880
Abdominal Visceral Fat	**50**	**0.35**	**0.012**	**49**	**0.34**	**0.024**	49	0.06	0.702

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and change in weight. Significant values after Hochberg correction represented in **bold**.

Table S6A: Partial Pearson's correlations between Percent Change in Positional AHI and Percent Change in Airway Size among Patients with OSA†

Airway Sizes	Supine AHI						Non-Supine AHI					
	Covariates Only			Covariates and Weight Change			Covariates Only			Covariates and Weight Change		
	N	rho‡	p	N	rho§	p	N	rho‡	p	N	rho§	p
RP Airway Volume	36	0.13	0.490	36	0.10	0.610	47	0.00	0.988	47	0.03	0.831
RP Cross Sectional Area	36	0.11	0.548	36	0.06	0.734	47	-0.05	0.771	47	0.03	0.832
RP Minimum Area	36	-0.01	0.958	36	-0.00	0.997	47	-0.07	0.669	47	-0.08	0.593
RP Minimum AP Distance	36	-0.11	0.538	36	-0.06	0.746	47	0.18	0.236	47	-0.02	0.923
RP Minimum Lateral Distance	36	0.08	0.652	36	0.04	0.814	47	-0.20	0.196	47	0.06	0.727
RG Airway Volume	35	-0.09	0.638	35	-0.10	0.583	45	0.12	0.463	45	0.06	0.696
RG Cross Sectional Area	33	-0.02	0.901	33	-0.04	0.826	43	0.33	0.041	43	0.25	0.138
RG Minimum Area	34	0.30	0.111	34	0.22	0.246	44	-0.31	0.055	44	0.15	0.364
RG Minimum AP Distance	33	-0.04	0.817	33	-0.03	0.879	44	0.22	0.166	44	0.12	0.457
RG Minimum Lateral Distance	33	-0.18	0.337	33	-0.19	0.339	44	0.09	0.584	44	0.14	0.387

†Analyses restricted to subsets of patients with specific positional AHI ≥ 5 events/hour at baseline; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S6B: Partial Pearson's Correlations between Percent Change in Positional AHI and Percent Change in Soft Tissues among Patients with OSA

Soft Tissue Volumes	Supine AHI						Non-Supine AHI					
	Covariates Only			Covariates and Weight Change			Covariates Only			Covariates and Weight Change		
	N	rho‡	p	N	rho§	p	N	rho‡	p	N	rho§	p
Combined Soft Tissue	35	-0.11	0.568	35	-0.10	0.614	47	0.16	0.307	47	-0.11	0.498
Soft Palate	36	0.22	0.220	36	0.21	0.261	47	-0.11	0.482	47	-0.15	0.350
Genioglossus	36	-0.18	0.334	36	-0.18	0.320	47	0.08	0.608	47	-0.01	0.956
Other Tongue	36	-0.02	0.914	36	-0.02	0.932	47	0.01	0.927	47	-0.11	0.473
Tongue Fat	31	0.22	0.260	31	0.36	0.070	**36**	**0.59**	**0.0004**	36	0.23	0.206
Total Tongue	36	-0.12	0.520	36	-0.13	0.493	47	0.11	0.503	47	-0.07	0.654
Epiglottis	35	-0.00	0.987	35	0.01	0.955	47	0.02	0.878	47	0.19	0.233
Fat Pads	35	-0.24	0.197	35	-0.26	0.160	47	0.20	0.194	47	0.07	0.668
Pterygoid	36	-0.06	0.734	36	0.00	0.980	47	0.10	0.514	47	-0.11	0.483
RP Lateral Walls	36	0.04	0.813	36	0.08	0.653	47	0.28	0.071	47	0.15	0.358
RG Lateral Walls	36	-0.01	0.939	36	0.00	0.982	47	0.01	0.968	47	-0.21	0.186
Total Lateral Walls	36	0.02	0.919	36	0.08	0.687	47	0.18	0.251	47	-0.07	0.669

†Analyses restricted to subsets of patients with specific positional AHI ≥ 5 events/hour at baseline; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S6C: Partial Pearson's Correlations between Percent Change in Positional AHI and Percent Change in Abdominal Fat Measures among Patients with OSA

Abdominal Fat Volume	Supine AHI						Non-Supine AHI					
	Covariates Only			Covariates and Weight Change			Covariates Only			Covariates and Weight Change		
	N	rho‡	p	N	rho§	p	N	rho‡	p	N	rho§	p
Abdominal Total Fat	25	0.38	0.090	25	-0.02	0.946	39	0.37	0.031	39	-0.03	0.874
Abdominal Subcutaneous Fat	25	0.34	0.132	25	0.02	0.939	39	0.36	0.032	39	0.00	0.988
Abdominal Visceral Fat	25	0.39	0.077	25	-0.14	0.566	39	0.29	0.089	39	-0.09	0.595

†Analyses restricted to subsets of patients with specific positional AHI ≥ 5 events/hour at baseline; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

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Effect of Weight Loss on Upper Airway Anatomy and the Apnea Hypopnea Index: The Importance of Tongue Fat

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SUPPLEMENTAL METHODS

Subjects

The study used a prospective design of persons with obesity and sleep apnea and who presented for either bariatric surgery or a lifestyle modification intervention for their obesity. Study participants were recruited from the Penn Center for Sleep Disorders outpatient practice and from patients seeking weight-loss treatment at the University of Pennsylvania's Bariatric Surgery Program or a lifestyle modification intervention at the Center for Weight and Eating Disorders. The study was approved by the University of Pennsylvania Institutional Review Board for human studies (protocols 808496 and 809398), and written informed consent was obtained from all participants. All participants were greater than 18 years old and had an apnea-hypopnea index (AHI) ≥10 events/hour as determined by polysomnography which were the criteria used in our previous study examining tongue fat in controls and apneics without weight loss (1). Exclusion criteria included inability to undergo upper airway and abdominal magnetic resonance imaging (MRI) or pregnancy. Participants underwent an MRI and in-laboratory sleep study before beginning weight loss treatment. Participants returned after 6 months for a follow-up MRI and in-laboratory sleep study.

Medical and Surgical Weight Loss Protocols

Participants undergoing a lifestyle modification program for weight loss (n=49) or bariatric surgery (n=18) were invited to participate. Patients undergoing bariatric surgery had gastric sleeve procedure (n=8), gastric bypass (Roux-en-Y) (n=9) or gastric banding (n=1). The lifestyle modification program was based upon the Diabetes Prevention Program and designed to promote a weight loss of 5-10% of initial body weight through caloric restriction, increased physical activity, and behavioral modification strategies (2). The calorie goals were 1200-1500 kcal/day for those who weighed <250 pounds, and 1500-1800 kcal/day for those whose weight exceeded 250 pounds. Dietary composition was aligned with that recommended by the NHLBI guidelines on the treatment of obesity (2) and included recommendations to consume <7% saturated fat, <10% polyunsaturated fat, <20% monounsaturated fats, 25-30% total fat, 50-60% carbohydrates, and 15% protein. Participants were prescribed a program of physical activity consisting of walking 4 times per week, starting at 10 minutes per session and building to 30 minutes per session over a 12-week period. Participants had 30-minute individual sessions with a registered dietitian with extensive experience in behavioral weight control counseling. Each patient received 24 weekly individual counseling sessions during the 6-month treatment period. Participants were instructed on how to comply with the dietary prescription and in traditional behavioral methods of weight control such as self-monitoring, stimulus control, slow eating, and related behaviors.

Magnetic Resonance Imaging (MRI)

Upper airway and abdominal MRI studies were acquired using a 1.5 Tesla MAGNETOM Espree scanner (Siemens Medical Systems, Malvern, PA), as described in our previous publications (1, 3-5). Three different imaging sequences were utilized as reported in our previous publications: 1) T1-weighted spin echo imaging (for airway and surrounding upper airway soft tissue structures) (3, 5); 2) Dixon imaging for tongue fat measurements (1); and 3) T1-weighted gradient recalled echo imaging for abdominal fat imaging (4).

Analysis of the Upper Airway, Surrounding Soft Tissue Structures and Abdominal Fat

The MRI analysis was performed twice (baseline and after 6 months) and split into 3 different domains: 1) *upper airway analysis* (airway volume, average cross-sectional area, minimum airway area, minimum anterior-posterior distance and minimum lateral distance) in the retropalatal (RP) and retroglossal (RG) regions; 2) *volumetric analysis of the upper airway soft tissues structures* (tongue, tongue fat, soft palate, parapharyngeal fat pads, lateral walls, pterygoids, epiglottis, and combined soft tissue volume [equal to the sum of these structures]); and 3) *abdominal fat volumes* (total, subcutaneous and visceral). As previously described, Amira 4.1.2 image analysis software (Visage Imaging, San Diego, CA), was utilized to quantify the upper airway, the surrounding soft tissue structures(1, 5, 6), tongue fat (1) and abdominal subcutaneous/visceral fat (4). Amira is a software program that allows the technician to segment the upper airway soft tissues structures using thresholding based on grayscale of the specific tissue (tongue, pharyngeal lateral walls, parapharyngeal fat pads, etc). Amira requires the technician to outline the pharyngeal structures. The technician was blinded to the pre-post-weight loss status of the patients and performed the analysis in the same manner in each subject. We have shown that our upper airway soft tissue and tongue fat analysis measurements are reproducible and accurate(1, 5). Accuracy of our fat volume estimates have been assessed previously (1) by performing Dixon MRI of a hamburger and steak before and after injection of a known volume of fat (lard) into the tissue (6 cc added to the steak and 3 cc added to the hamburger) and comparing the results to the known quantity.

Although fat-weighted Dixon images provide a reproducible objective measure adipose tissue, the boundaries of soft tissues with Dixon imaging are not as distinct in comparison to the standard spin-echo images (1). Therefore the boundaries of the tongue on each MR axial slice was determined on standard axial T1 spin-echo MR images, and then superimposed onto the axial Dixon fat-only MR images in order to select all fat within that region.. The grayscale setting to segment tongue fat was chosen based on the grayscale intensity of the surrounding subcutaneous and neck fat allowing us to standardize the tongue fat intensity across all subjects (1). We have shown that this analysis technique for tongue fat is highly reproducible (1). The technician performing these MRI analyses was blinded to polysomnography results and the pre-post weight loss state of each subject.

Reproducibility Assessment

To understand the reproducibility of MRI analyses, we compared measurements quantified from MRIs performed 6 months apart in an available sample of 17 individuals (n=13 OSA, 4 non-OSA) that were weight stable over the follow-up period (defined as follow-up weight within 2.5% of baseline weight). Using these repeated measurements, we calculated the intraclass correlation coefficient (ICC) for each of our anatomy measures of interest. As suggested by Landis and Koch (7), ICC value ranges can be used to qualitatively assess level of reproducibility as *poor* (<0.00), *slight* (0.00-0.20), *fair* (0.21-0.40), *moderate* (0.41-0.60), *substantial* (0.61-0.80) and *almost perfect* (0.81-1.00).

Statistical Analysis

Data are summarized using means and standard deviations (continuous) or frequencies and percentages (categorical). To summarize changes over the follow-up period, we calculated subject-specific percentage and absolute change scores as follow-up minus baseline values. Primary analyses examining the relationship between weight loss and anatomic changes were performed using Pearson's correlations, unadjusted and controlling for relevant baseline covariates (age, sex, race, height and AHI). Associations between change in upper airway anatomy (or weight) and change in AHI were performed in a similar manner. These analyses were performed unadjusted, controlling for clinical covariates, and further adjusted for percentage change in weight to assess whether individual structures were associated with changes in AHI independent of change in weight. As a complementary analysis, we also quantified and compared change scores in patients that lost at least 2.5% weight and those that were stable or gained weight. Comparisons between groups were performed using T-tests or linear regression models with and without covariate adjustment. In addition to the above covariates, baseline values of the variable of interest were included in models comparing absolute change between groups. Significance of within group changes were assessed with paired T-tests. Where presented, statistical comparisons of correlation coefficients were performed by deriving a non-parametric p-value based on the observed distribution of differences in correlations from 1,000 bootstrapped samples (8, 9).

To test whether associations between percent weight change and percent AHI improvements are mediated by specific upper airway or soft tissue anatomy percent changes, we utilized mediation modeling (10). Mediation analyses were conducted by PROCESS, a conditional process modeling program that utilizes an ordinary least squares path analytical framework to test for both direct and indirect effects (11). To comprehensively examine upper airway mediators, we first tested all putative mediators individually using single mediator models. Based on the results of these single mediator models, we then created a combined, parallel multiple mediator model including any significant individual mediators to examine the most influential mediators in the presence of all possible mediators (see **Figure S1** for hypothesized mediation models). Bias-corrected 95% confidence intervals (CIs) were estimated using bootstrapping (n=5000 samples) to verify indirect (mediating) effects; estimates are presented unstandardized and standardized (to allow direct comparison of indirect effects across proposed mediators). The indirect effect was considered significant and mediation demonstrated if this confidence interval did not contain zero. (10, 12). This approach computes more accurate confidence intervals of indirect effects than other commonly used methods (10) and provides higher power while maintaining control over the Type I error rate (13). Baseline age, sex, race, and height were controlled for all mediation models.

We utilized a domain-specific Hochberg correction (14-16) to control for multiple comparisons when determining statistical significance of associations with individual upper airway anatomical measures. As described by Hochberg and Benjamini (15), for a given set of *m* hypotheses:

> *This procedure starts by examining the largest p-value $P_{(m)}$. If $P_{(m)} \leq \alpha$, then $H_{(m)}$ and all other hypotheses are rejected. If not, $H_{(m)}$ is not rejected and one proceeds to compare $P_{(m-1)}$ with $\alpha/2$. If the former is smaller, then $H_{(m-1)}$ and all hypotheses with smaller p-values are rejected. Generally, one proceeds from highest to lower p-values, retaining $H_{(i)}$ if its p-value satisfies $P_{(i)}$*

> α/(m - i + 1). One stops the procedure at the first ordered hypothesis when that inequality is reversed. This hypothesis is rejected and so are all hypotheses with lower or equal p-values.

In addition to this approach for determining statistically significant results, any unadjusted $p<0.05$ was considered nominally significant evidence. Significance in multivariate models was assessed using the confidence interval approach, detailed above. Analyses were conducted using Stata, Version 14 (StataCorp LP, College Station, TX), SAS Software Version 9.4 (SAS Institute Inc., Cary, NC) and SPSS 24 (IBM Corp., Armonk, NY).

Our sample size of 67 apneics provided adequate power (80%) for detecting correlations of 0.34 at a nominal level of significance ($p<0.05$). Similarly, in analyses comparing apneics who lost weight (n=47) to those who were weight stable or gained weight (n=20), we had at least 80% power to detect standardized differences of 0.76. These correspond to moderate or large effects as defined by Cohen (17). Thus, the study was powered for meaningful effect sizes. Non-significant associations for smaller effect sizes should be interpreted with some caution, as it is possible that these represent real associations that the current sample is underpowered to declare significant (e.g., false negatives).

SUPPLEMENTAL RESULTS

Patient characteristics of weight loss groups

Demographic characteristics of the sample are shown in **Table 1**. Forty-seven (70.1%) patients lost ≥2.5% body weight (average change -14.5±8.5% [p<0.0001]), compared to 20 (29.9%) that were weight stable or gained weight (average change 2.3±4.5% [p=0.036]). Patients who lost weight had a significant AHI reduction (-23.3±21.9; p<0.0001), compared to no change in those that did not (p=0.856).

Comparison of weight loss interventions

All participant in the bariatric surgery arm (n=18) lost at least 2.5% body weight, while in those undergoing lifestyle modification a total of 29 (59.2%) lost at least 2.5% body weight and 20 (40.8%) were weight stable or gained weight. Among participants that lost weight, those that underwent bariatric surgery had greater weight loss than those that underwent intensive lifestyle modification (-19.2±6.8% vs. -11.6±8.2%, p=0.002). Relatedly, there was greater improvement in AHI among patients who lost weight with bariatric surgery than those who lost weight with intensive lifestyle modification (-73.8±17.8% vs. -49.6±36.5%, p=0.004).

Changes in Upper Airway Anatomy in Weight Loss and Weight Stable/Gain Patients

Airway Sizes

Secondary comparisons of percent changes in airway size between weight loss and weight stable/gain patients are shown in **Table S32A**. Patients who lost ≥2.5% weight showed increased retropalatal airway volume (p=0.007), cross-sectional area (p=0.001), minimum area (p=0.003) and minimum lateral distance (p=0.001), as well as a large increase in retroglossal minimum area (p=0.001). After covariate adjustment, differences in the percentage changes in the RP minimum lateral distance (p=0.020) and RG minimum area (p=0.023) were nominally different between weight loss groups

(**Table S3~~2~~A**). Similar results were observed when comparing absolute changes (**Table S4~~3~~A**), although the increased RG minimum area was nominal (p=0.032) among the weight loss group. There was also a significant absolute decrease in RP minimum AP distance (p=0.003) among the weight loss group. After adjustment, differences in RP minimum AP (p=0.022) and lateral (p=0.033) distance remained nominally different between groups.

Soft Tissue Volumes

Supporting results observed in correlational analysis, most soft tissue measures showed significant decreases in volume among patients with OSA who lost weight (**Table S3~~2~~B**), including combined soft tissue (p<0.0001), genioglossus (p=0.001), tongue fat (p<0.0001), total tongue (p=0.009), fat pads (p<0.0001), pterygoid (p<0.0001), RP lateral walls (p<0.0001) and total lateral walls (p<0.0001). Compared to patients without weight loss, there were significant differences in the change in tongue fat volume (p<0.0001), pterygoid volume (p=0.001) and total lateral wall volume (p=0.001). Differences remained significant controlling for covariates (**Table S3~~2~~B**). Similar results were observed for absolute changes (**Tables S4~~3~~B**); in adjusted analyses, we also observed a significant difference in the absolute change in fat pad volume between groups (p=0.004).

Abdominal Fat Volumes

Among patients who lost ≥2.5% weight, there were large percentage reductions in each measurement (**Table S3~~2~~C**), compared to no significant changes in those who did not lose weight. Differences between groups were statistically significant in adjusted analyses. Similar results were seen for absolute changes (**Table S4~~3~~C**), with significant differences between groups in adjusted analysis.

Associations between Changes in Tongue Fat and Abdominal Fat

Given correlations between percent change in weight and percent changes in tongue and abdominal fat volumes, we assessed the correlations between tongue and abdominal fat changes. In covariate adjusted analyses, percentage change in tongue fat was positively associated with percentage changes in total (partial rho = 0.48, p=0.005), subcutaneous (partial rho = 0.40, p=0.021), and visceral (partial rho = 0.55, p=0.0009) fat volumes. Correlations between the percentage changes in tongue fat and both total abdominal fat (p=0.034) and subcutaneous abdominal fat (p=0.026), but not visceral abdominal fat (p=0.154), were smaller than the correlation between percentage changes in tongue fat and weight. Thus, there is some evidence of a stronger relationship of percent changes in tongue fat with changes in weight.

Figure S1. *Illustration of hypothesized single and multiple mediation models.* The hypothesized mediation models examined through conditional process analysis are illustrated. In particular, the direct effect of percent change in weight on percentage change in AHI (*path c*) is shown in the top schematic. The middle schematic shows the evaluated single mediator model of mediation by an individual upper airway variable, including the relationship between weight percent change and anatomy (*path a*), between anatomy and percentage change in AHI (*path b*) and the remaining direct effect of weight percent change on AHI percent change (*path c'*). Finally, the single mediator model is extended to show the hypothesized multiple mediator model, including *N* single mediators with specific individual a_N and b_N path effects.

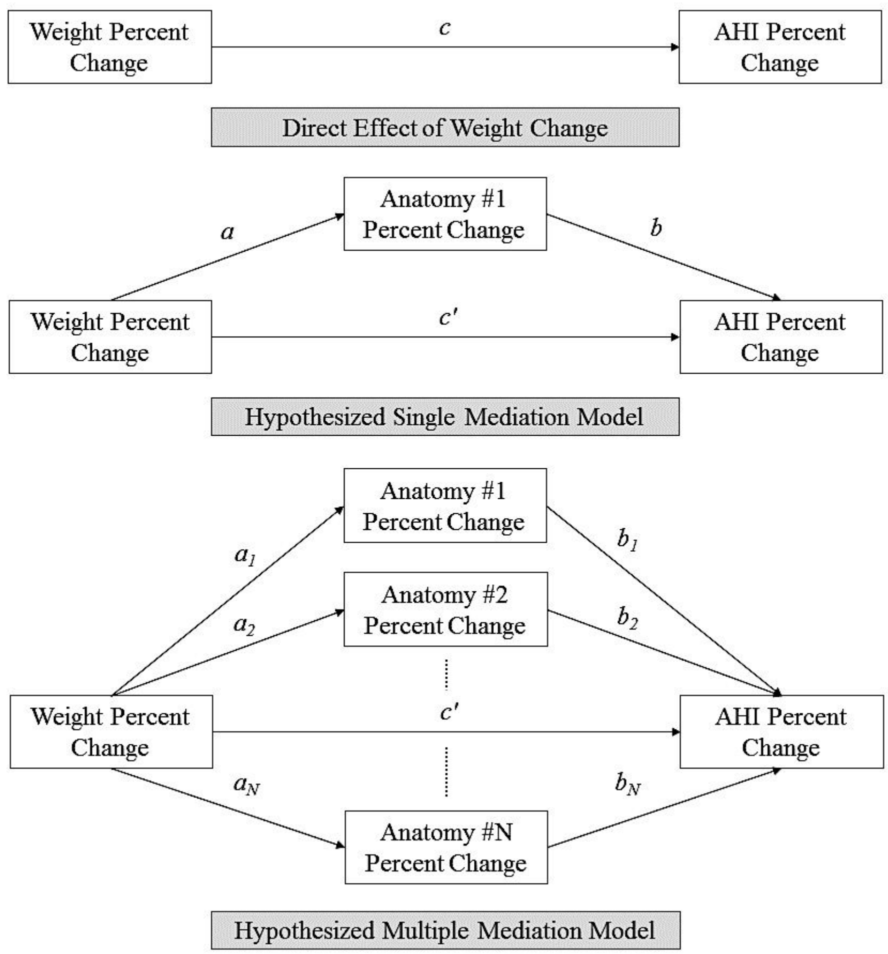

Table S1: Intraclass Correlation Coefficients for MRI Variables Among a Weight Stable Population

Measure	**Weight Stable Participants**	
	N	**ICC†**
RP Airway Volume	17	0.777
RP Cross Sectional Area	17	0.808
RP Minimum Area	17	0.935
RP Minimum AP Distance	17	0.701
RP Minimum Lateral Distance	17	0.889
RG Airway Volume	14	0.844
RG Cross Sectional Area	14	0.856
RG Minimum Area	15	0.849
RG Minimum AP Distance	15	0.746
RG Minimum Lateral Distance	15	0.829
Total Soft Tissue Volume	17	0.944
Soft Palate Volume	17	0.779
Genioglossus Volume	17	0.922
Other Tongue Volume	17	0.721
Tongue Fat Volume	11	0.979
Total Tongue Volume	17	0.946
Epiglottis Volume	17	0.750
Fat Pads Volume	17	0.353
Pterygoid Volume	17	0.694
RP Lateral Walls Volume	17	0.911
RG Lateral Walls Volume	17	0.758
Total Lateral Walls Volume	17	0.910
Abdominal Total Fat Volume	14	0.489
Abdominal Subcutaneous Fat Volume	14	0.536
Abdominal Visceral Fat Volume	14	0.751

†Intraclass correlation coefficient (ICC) presented as a measure of reproducibility of the indicated measure over time among weight stable individuals; As suggested by Landis and Koch (23), ICC value ranges can be used to qualitatively assess level of agreement as *poor* (<0.00), *slight* (0.00-0.20), *fair* (0.21-0.40), *moderate* (0.41-0.60), *substantial* (0.61-0.80) and *almost perfect* (0.81-1.00). Abbreviations: ICC = intraclass correlation coefficient; RP = retropalatal; RG = retroglossal

Table S2~~1~~A: Pearson's Correlations between Absolute Change in Weight and Absolute Change in Airway Size among Patients with OSA

Airway Sizes	Unadjusted			Adjusted		
	N	rho†	p	N	rho‡	p
RP Airway Volume	64	-0.16	0.211	63	-0.21	0.110
RP Cross Sectional Area	64	-0.21	0.103	63	-0.27	0.037
RP Minimum Area	64	-0.03	0.789	63	-0.05	0.688
RP Minimum AP Distance	**64**	**0.36**	**0.003**	**63**	**0.49**	**0.0001**
RP Minimum Lateral Distance	64	-0.33	0.008	**63**	**-0.40**	**0.002**
RG Airway Volume	62	0.08	0.535	61	0.11	0.434
RG Cross Sectional Area	60	0.06	0.662	59	0.04	0.761
RG Minimum Area	61	-0.01	0.934	60	-0.13	0.335
RG Minimum AP Distance	60	0.26	0.046	59	0.24	0.078
RG Minimum Lateral Distance	60	-0.03	0.842	59	-0.04	0.757

†Unadjusted Pearson's linear correlations; ‡Partial Pearson's correlation adjusted for age, gender, race, AHI and height. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S2~~1~~B: Pearson's Correlations between Absolute Change in Weight and Absolute Change in Soft Tissue Volumes among Patients with OSA

Soft Tissue Volumes	Unadjusted			Adjusted		
	N	rho†	p	N	rho‡	p
Combined Soft Tissue	63	0.09	0.488	62	0.10	0.456
Soft Palate	64	-0.12	0.338	63	-0.12	0.363
Genioglossus	64	-0.03	0.825	63	-0.03	0.799
Other Tongue	64	-0.06	0.649	63	-0.03	0.811
Tongue Fat	**52**	**0.47**	**0.0004**	**51**	**0.48**	**0.001**
Total Tongue	64	-0.06	0.639	63	-0.05	0.727
Epiglottis	63	0.04	0.737	62	0.06	0.632
Fat Pads	63	0.06	0.631	62	0.04	0.787
Pterygoid	**64**	**0.37**	**0.002**	63	0.37	0.005
RP Lateral Walls	64	0.16	0.212	63	0.15	0.267
RG Lateral Walls	64	0.17	0.171	63	0.22	0.094
Total Lateral Walls	64	0.24	0.054	63	0.28	0.035

†Unadjusted Pearson's linear correlations; ‡Partial Pearson's correlation adjusted for age, gender, race, AHI and height. Significant values represented in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S2~~1~~C: Pearson's Correlations between Absolute Change in Weight and Absolute Change in Abdominal Fat Measures among Patients with OSA

Abdominal Fat Volume	Unadjusted			Adjusted		
	N	rho†	p	N	rho‡	p
Abdominal Total Fat	**51**	**0.57**	**<0.0001**	**50**	**0.58**	**<0.0001**
Abdominal Subcutaneous Fat	**51**	**0.56**	**<0.0001**	**50**	**0.57**	**<0.0001**
Abdominal Visceral Fat	**51**	**0.49**	**0.0003**	**50**	**0.54**	**0.0001**

†Unadjusted Pearson's linear correlations; ‡Partial Pearson's correlation adjusted for age, gender, race, AHI and height. Significant values represented in **bold**.

Table S3~~2~~A: Percent Changes in Airway Size in Patients with OSA based on weight change

Airway Sizes	Weight Stable/Gain			Weight Loss			p_{unadj}‡	p_{adj}§
	N	Mean ± SD	p†	N	Mean ± SD	p†		
RP Airway Volume	19	13.8 ± 47.0	0.216	**45**	**21.5 ± 51.3**	**0.007**	0.579	0.512
RP Cross Sectional Area	19	15.4 ± 42.0	0.127	**45**	**24.4 ± 47.5**	**0.001**	0.475	0.374
RP Minimum Area	19	17.8 ± 58.1	0.197	**45**	**55.9 ± 116.7**	**0.003**	0.088	0.158
RP Minimum AP Distance	19	9.7 ± 42.6	0.332	45	-3.1 ± 77.3	0.789	0.399	0.426
RP Minimum Lateral Distance	19	1.1 ± 30.6	0.879	**45**	**33.8 ± 65.6**	**0.001**	0.009	0.020
RG Airway Volume	17	19.6 ± 29.6	0.015	45	7.8 ± 35.7	0.149	0.230	0.317
RG Cross Sectional Area	17	19.6 ± 36.4	0.042	43	6.3 ± 28.7	0.160	0.140	0.197
RG Minimum Area	17	21.7 ± 57.1	0.136	**44**	**424.6 ± 778.8**	**0.001**	**0.001**	0.023
RG Minimum AP Distance	17	8.1 ± 40.4	0.421	43	7.0 ± 48.2	0.343	0.938	0.998
RG Minimum Lateral Distance	17	13.3 ± 44.3	0.233	43	16.3 ± 54.4	0.055	0.840	0.668

†p-value from paired T-test examining significance of within group change; ‡p-value from T-test comparing changes between weight loss and weight stable/gain; §p-value adjusted for age, sex, race, AHI and height. Significant values after Hochberg correction represented in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S3~~2~~B: Percent Changes in Soft Tissue Volumes in Patients with OSA based on weight change

Soft Tissue Volumes	Weight Stable/Gain			Weight Loss			p_{unadj}‡	p_{adj}§
	N	Mean ± SD	p†	N	Mean ± SD	p†		
Combined Soft Tissue	19	-3.3 ± 6.3	0.035	**44**	**-7.4 ± 9.0**	**<0.0001**	0.077	0.057
Soft Palate	19	-8.6 ± 22.7	0.115	45	-0.1 ± 24.0	0.986	0.190	0.197
Genioglossus	19	-3.0 ± 7.3	0.092	**45**	**-5.1 ± 10.0**	**0.001**	0.409	0.318
Other Tongue	19	-2.4 ± 25.0	0.682	45	-0.8 ± 28.4	0.859	0.828	0.951
Tongue Fat	13	6.8 ± 11.5	0.055	**39**	**-19.9 ± 17.8**	**<0.0001**	**<0.0001**	**<0.0001**
Total Tongue	19	-3.7 ± 6.5	0.023	**45**	**-4.6 ± 11.4**	**0.009**	0.692	0.577
Epiglottis	19	14.7 ± 38.2	0.111	44	3.6 ± 40.1	0.557	0.309	0.211
Fat Pads	19	-15.9 ± 26.0	0.016	**44**	**-28.3 ± 22.2**	**<0.0001**	0.058	0.078
Pterygoid	19	1.5 ± 16.9	0.704	**45**	**-12.0 ± 13.4**	**<0.0001**	**0.001**	**0.004**
RP Lateral Walls	19	-2.6 ± 16.4	0.504	**45**	**-11.9 ± 15.1**	**<0.0001**	0.032	0.022
RG Lateral Walls	19	14.3 ± 22.0	0.011	45	-2.8 ± 24.2	0.441	0.010	0.020
Total Lateral Walls	19	3.4 ± 14.8	0.333	**45**	**-9.3 ± 11.8**	**<0.0001**	**0.001**	**0.001**

†p-value from paired T-test examining significance of within group change; ‡p-value from T-test comparing changes between weight loss and weight stable/gain; §p-value adjusted for age, sex, race, AHI and height. Significant values after Hochberg correction represented in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S3~~2~~C: Percent Changes in Abdominal Fat Volumes in Patients with OSA based on weight change

Abdominal Fat Volume	Weight Stable/Gain			Weight Loss			p_{unadj}‡	p_{adj}§
	N	Mean ± SD	p†	N	Mean ± SD	p†		
Abdominal Total Fat	15	-0.1 ± 38.4	0.991	**36**	**-21.1 ± 17.8**	**<0.0001**	0.059	**0.013**
Abdominal Subcutaneous Fat	15	-1.4 ± 36.0	0.883	**36**	**-19.4 ± 18.2**	**<0.0001**	0.084	**0.024**
Abdominal Visceral Fat	15	9.5 ± 64.4	0.576	**36**	**-25.9 ± 21.6**	**<0.0001**	0.055	**0.007**

†p-value from paired T-test examining significance of within group change; ‡p-value from T-test comparing changes between weight loss and weight stable/gain; §p-value adjusted for age, sex, race, AHI and height. Significant values after Hochberg correction represented in **bold.**

Table S43A: Absolute Changes in Airway Size in Patients with OSA based on weight change

Airway Sizes	**Weight Stable/Gain**			**Weight Loss**			**p_{unadj}‡**	**p_{adj}§**
	N	**Mean ± SD**	**p†**	**N**	**Mean ± SD**	**p†**		
RP Airway Volume, mm³	19	103.8 ± 1773.5	0.802	45	377.2 ± 1434.5	0.085	0.519	0.754
RP Cross Sectional Area, mm²	19	32.0 ± 134.3	0.312	45	53.1 ± 125.6	0.007	0.551	0.632
RP Minimum Area, mm²	19	3.3 ± 23.1	0.547	45	13.4 ± 39.1	0.027	0.205	0.232
RP Minimum AP Distance, mm	19	0.47 ± 3.23	0.533	**45**	**-3.54 ± 7.52**	**0.003**	**0.004**	0.022
RP Minimum Lateral Distance, mm	19	-0.26 ± 2.42	0.642	**45**	**2.24 ± 4.79**	**0.003**	0.008	0.033
RG Airway Volume, mm³	17	1030.8 ± 1823.3	0.033	45	-25.9 ± 2395.0	0.943	0.105	0.286
RG Cross Sectional Area, mm²	17	88.7 ± 242.7	0.151	43	18.5 ± 195.6	0.539	0.247	0.379
RG Minimum Area, mm²	17	16.9 ± 81.3	0.404	44	39.6 ± 118.6	0.032	0.471	0.848
RG Minimum AP Distance, mm	17	0.26 ± 4.42	0.813	43	-0.52 ± 4.49	0.456	0.548	0.220
RG Minimum Lateral Distance, mm	17	0.23 ± 5.57	0.866	43	0.72 ± 6.10	0.443	0.776	0.944

†p-value from paired T-test examining significance of within group change; ‡p-value from T-test comparing changes between weight loss and weight stable/gain; §p-value adjusted for age, sex, race, AHI, height and baseline airway size. Significant values after Hochberg correction represented in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S43B: Absolute Changes in Soft Tissue Volumes in Patients with OSA based on weight change

Soft Tissue Volumes (mm³)	**Weight Stable/Gain**			**Weight Loss**			**p_{unadj}‡**	**p_{adj}§**
	N	**Mean ± SD**	**p†**	**N**	**Mean ± SD**	**p†**		
Combined Soft Tissue	19	-6959.9 ± 11999.6	0.021	**44**	**-15100.3 ± 19816.8**	**<0.0001**	0.050	0.054
Soft Palate	19	-894.0 ± 1866.5	0.051	45	-191.4 ± 1746.8	0.466	0.155	0.381
Genioglossus	19	-3014.8 ± 7137.1	0.082	**45**	**-5284.8 ± 10333.8**	**0.001**	0.387	0.306
Other Tongue	19	-2286.4 ± 9895.2	0.327	45	-1197.6 ± 10204.6	0.435	0.695	0.444
Tongue Fat	13	1864.4 ± 3173.6	0.056	**39**	**-5536.7 ± 5110.1**	**<0.0001**	**<0.0001**	**0.0001**
Total Tongue	19	-5301.2 ± 8809.8	0.017	**45**	**-6482.4 ± 16082.5**	**0.010**	0.708	0.512
Epiglottis	19	124.3 ± 544.3	0.333	44	-33.5 ± 613.7	0.719	0.337	0.028
Fat Pads	19	-1504.5 ± 2276.3	0.010	**44**	**-2219.8 ± 1927.1**	**<0.0001**	0.206	**0.004**
Pterygoid	19	232.8 ± 4043.8	0.805	**45**	**-3149.4 ± 3444.5**	**<0.0001**	**0.001**	**0.0002**
RP Lateral Walls	19	-684.4 ± 2108.1	0.174	**45**	**-1795.2 ± 2381.8**	**<0.0001**	0.083	0.016
RG Lateral Walls	19	1067.2 ± 2065.4	0.037	45	-807.4 ± 3044.4	0.082	0.017	0.040
Total Lateral Walls	19	382.8 ± 2808.7	0.560	**45**	**-2602.7 ± 3566.0**	**<0.0001**	**0.002**	**0.001**

†p-value from paired T-test examining significance of within group change; ‡p-value from T-test comparing changes between weight loss and weight stable/gain; §p-value adjusted for age, sex, race, AHI, height and baseline soft tissue volume. Significant values after Hochberg correction represented in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S43C: Absolute Changes in Abdominal Fat Volumes in Patients with OSA based on weight change

Abdominal Fat Volume (mm³)	**Weight Stable/Gain**			**Weight Loss**			**p_{unadj}‡**	**p_{adj}§**
	N	**Mean ± SD**	**p†**	**N**	**Mean ± SD**	**p†**		
Abdominal Total Fat	15	-1337194 ± 6041271	0.406	**36**	**-3825576 ± 4108429**	**<0.0001**	0.094	**0.002**
Abdominal Subcutaneous Fat	15	-1019828 ± 4808678	0.425	**36**	**-2898336 ± 3418169**	**<0.0001**	0.120	**0.006**
Abdominal Visceral Fat	15	-317367 ± 1441512	0.408	**36**	**-927240 ± 958480**	**<0.0001**	0.082	**0.001**

†p-value from paired T-test examining significance of within group change; ‡p-value from T-test comparing changes between weight loss and weight stable/gain; §p-value adjusted for age, sex, race, AHI, height and baseline abdominal fat volume. Significant values after Hochberg correction represented in **bold.**

Table S54A: Correlations between Absolute Change in AHI and Absolute Change in Airway Size among Patients with OSA

Airway Sizes	Unadjusted			Adjusted Results					
				Covariates Only†			Covariates and Weight Change‡		
	N	rho†	p	N	rho‡	p	N	rho§	p
RP Airway Volume	63	0.15	0.241	62	0.08	0.576	62	0.24	0.077
RP Cross Sectional Area	63	0.12	0.347	62	0.05	0.737	62	0.24	0.067
RP Minimum Area	63	-0.04	0.726	62	-0.05	0.712	62	-0.03	0.833
RP Minimum AP Distance	63	0.26	0.038	62	0.30	0.022	62	0.04	0.762
RP Minimum Lateral Distance	63	-0.17	0.173	62	-0.18	0.183	62	0.06	0.681
RG Airway Volume	61	-0.12	0.371	60	-0.14	0.292	60	-0.23	0.090
RG Cross Sectional Area	59	0.02	0.867	58	0.00	0.973	58	-0.02	0.873
RG Minimum Area	60	-0.06	0.657	59	-0.07	0.601	59	0.00	0.977
RG Minimum AP Distance	59	0.12	0.347	58	0.16	0.249	58	0.01	0.944
RG Minimum Lateral Distance	59	-0.10	0.429	58	-0.23	0.102	58	-0.22	0.107

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction represented in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S54B: Correlations between Absolute Change in AHI and Absolute Change in Soft Tissues among Patients with OSA

Soft Tissue Volumes	Unadjusted			Adjusted Results					
				Covariates Only†			Covariates and Weight Change‡		
	N	rho†	p	N	rho‡	p	N	rho§	p
Combined Soft Tissue	62	0.09	0.482	61	0.05	0.698	61	0.01	0.913
Soft Palate	63	-0.06	0.625	62	-0.08	0.554	62	-0.01	0.944
Genioglossus	63	0.03	0.794	62	0.05	0.735	62	0.09	0.507
Other Tongue	63	-0.04	0.734	62	-0.07	0.618	62	-0.05	0.721
Tongue Fat	**51**	**0.47**	**0.001**	**50**	**0.47**	**0.001**	50	0.29	0.057
Total Tongue	63	-0.01	0.947	62	-0.02	0.901	62	0.03	0.846
Epiglottis	62	0.13	0.316	61	0.08	0.539	61	0.06	0.673
Fat Pads	62	0.09	0.501	61	0.09	0.483	61	0.09	0.505
Pterygoid	63	0.28	0.024	62	0.19	0.148	62	-0.02	0.903
RP Lateral Walls	63	0.30	0.018	62	0.25	0.061	62	0.21	0.120
RG Lateral Walls	63	-0.05	0.697	62	-0.10	0.448	62	-0.25	0.058
Total Lateral Walls	63	0.15	0.233	62	0.07	0.594	62	-0.08	0.574

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction represented in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S5~~4~~C: Correlations between Absolute Change in AHI and Absolute Change in Abdominal Fat Measures among Patients with OSA

Abdominal Fat Volume	Unadjusted			Adjusted Results					
				Covariates Only			Covariates and Weight Change		
	N	rho†	p	N	rho‡	p	N	rho§	p
Abdominal Total Fat	50	0.31	0.029	**49**	**0.34**	**0.021**	49	0.03	0.829
Abdominal Subcutaneous Fat	50	0.28	0.051	**49**	**0.33**	**0.027**	49	0.02	0.880
Abdominal Visceral Fat	**50**	**0.35**	**0.012**	**49**	**0.34**	**0.024**	49	0.06	0.702

†Unadjusted Pearson's linear correlation; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and change in weight. Significant values after Hochberg correction represented in **bold**.

Table S6~~5~~A: Partial Pearson's correlations between Percent Change in Positional AHI and Percent Change in Airway Size among Patients with OSA†

Airway Sizes	Supine AHI						Non-Supine AHI					
	Covariates Only			Covariates and Weight Change			Covariates Only			Covariates and Weight Change		
	N	rho‡	p	N	rho§	p	N	rho‡	p	N	rho§	p
RP Airway Volume	36	0.13	0.490	36	0.10	0.610	47	0.00	0.988	47	0.03	0.831
RP Cross Sectional Area	36	0.11	0.548	36	0.06	0.734	47	-0.05	0.771	47	0.03	0.832
RP Minimum Area	36	-0.01	0.958	36	-0.00	0.997	47	-0.07	0.669	47	-0.08	0.593
RP Minimum AP Distance	36	-0.11	0.538	36	-0.06	0.746	47	0.18	0.236	47	-0.02	0.923
RP Minimum Lateral Distance	36	0.08	0.652	36	0.04	0.814	47	-0.20	0.196	47	0.06	0.727
RG Airway Volume	35	-0.09	0.638	35	-0.10	0.583	45	0.12	0.463	45	0.06	0.696
RG Cross Sectional Area	33	-0.02	0.901	33	-0.04	0.826	43	0.33	0.041	43	0.25	0.138
RG Minimum Area	34	0.30	0.111	34	0.22	0.246	44	-0.31	0.055	44	0.15	0.364
RG Minimum AP Distance	33	-0.04	0.817	33	-0.03	0.879	44	0.22	0.166	44	0.12	0.457
RG Minimum Lateral Distance	33	-0.18	0.337	33	-0.19	0.339	44	0.09	0.584	44	0.14	0.387

†Analyses restricted to subsets of patients with specific positional AHI ≥ 5 events/hour at baseline; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S65B: Partial Pearson's Correlations between Percent Change in Positional AHI and Percent Change in Soft Tissues among Patients with OSA

Soft Tissue Volumes	**Supine AHI**						**Non-Supine AHI**					
	Covariates Only			**Covariates and Weight Change**			**Covariates Only**			**Covariates and Weight Change**		
	N	**rho‡**	**p**	**N**	**rho§**	**p**	**N**	**rho‡**	**p**	**N**	**rho§**	**p**
Combined Soft Tissue	35	-0.11	0.568	35	-0.10	0.614	47	0.16	0.307	47	-0.11	0.498
Soft Palate	36	0.22	0.220	36	0.21	0.261	47	-0.11	0.482	47	-0.15	0.350
Genioglossus	36	-0.18	0.334	36	-0.18	0.320	47	0.08	0.608	47	-0.01	0.956
Other Tongue	36	-0.02	0.914	36	-0.02	0.932	47	0.01	0.927	47	-0.11	0.473
Tongue Fat	31	0.22	0.260	31	0.36	0.070	**36**	**0.59**	**0.0004**	36	0.23	0.206
Total Tongue	36	-0.12	0.520	36	-0.13	0.493	47	0.11	0.503	47	-0.07	0.654
Epiglottis	35	-0.00	0.987	35	0.01	0.955	47	0.02	0.878	47	0.19	0.233
Fat Pads	35	-0.24	0.197	35	-0.26	0.160	47	0.20	0.194	47	0.07	0.668
Pterygoid	36	-0.06	0.734	36	0.00	0.980	47	0.10	0.514	47	-0.11	0.483
RP Lateral Walls	36	0.04	0.813	36	0.08	0.653	47	0.28	0.071	47	0.15	0.358
RG Lateral Walls	36	-0.01	0.939	36	0.00	0.982	47	0.01	0.968	47	-0.21	0.186
Total Lateral Walls	36	0.02	0.919	36	0.08	0.687	47	0.18	0.251	47	-0.07	0.669

†Analyses restricted to subsets of patients with specific positional AHI ≥ 5 events/hour at baseline; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

Table S65C: Partial Pearson's Correlations between Percent Change in Positional AHI and Percent Change in Abdominal Fat Measures among Patients with OSA

Abdominal Fat Volume	**Supine AHI**						**Non-Supine AHI**					
	Covariates Only			**Covariates and Weight Change**			**Covariates Only**			**Covariates and Weight Change**		
	N	**rho‡**	**p**	**N**	**rho§**	**p**	**N**	**rho‡**	**p**	**N**	**rho§**	**p**
Abdominal Total Fat	25	0.38	0.090	25	-0.02	0.946	39	0.37	0.031	39	-0.03	0.874
Abdominal Subcutaneous Fat	25	0.34	0.132	25	0.02	0.939	39	0.36	0.032	39	0.00	0.988
Abdominal Visceral Fat	25	0.39	0.077	25	-0.14	0.566	39	0.29	0.089	39	-0.09	0.595

†Analyses restricted to subsets of patients with specific positional AHI ≥ 5 events/hour at baseline; ‡Partial Pearson's correlation adjusted for age, gender, race and height; §Partial Pearson's correlation adjusted for age, gender, race, height and percent change in weight. Significant values after Hochberg correction shown in **bold**. Abbreviations: RP = retropalatal; RG = retroglossal

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November 14, 2019

Jadwiga A. Wedzicha, MD
Editor, *American Journal of Respiratory and Critical Care Medicine*

Reena Mehra, MD
Associate Editor, *American Journal of Respiratory and Critical Care Medicine*

Dear Drs. Wedzicha and Mehra,

We thank the Editors and Reviewers for their careful consideration of our manuscript entitled, *Effect of Weight Loss on Upper Airway Anatomy and the Apnea Hypopnea Index: The Importance of Tongue Fat*. Please find below our point-by-point responses to the critique raised during the review process, with noted edits to the manuscript and online supplement highlighted in the marked version.

The authors think that we have appropriately responded the critique, and thank the Reviewers and Editor again for their suggestions, which have served to improve the manuscript.

Thank you for your consideration of our revised submission.

Sincerely,

Richard J. Schwab
Professor of Medicine
Chief, Division of Sleep Medicine
University of Pennsylvania

Comments from Reviewer 1: None

Comments from Reviewer 2:

Comment R2.1: The authors have improved the writing of the manuscript and have adequately addressed the reviewers comments. There are still a lot of tables with a lot of information within them in the main manuscript. I would suggest that Table 1 could also be moved to the supplement.

Response R2.1: *We agree with the reviewer and moved Table 1 to the supplement. All the other tables in the main manuscript and supplement were re-numbered and appropriate changes were made to the text to reflect then table number changes.*