



Influence of reflux and central obesity on intercellular space diameter of esophageal squamous epithelium

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Abstract

Background: While central obesity increases gastroesophageal reflux (GER) by mechanically disrupting the anti-reflux barrier, limited data exist on pathways by which central obesity may potentiate esophageal injury by non-mechanical means. Obesity has been associated with an impaired epithelial intestinal barrier.

Objective: We aimed to assess the influence of central obesity and reflux on the squamous esophageal epithelial intercellular space diameter (ICSD).

Methods: The ICSD was measured using electron microscopy in esophageal biopsies from individuals who underwent ambulatory pH monitoring and endoscopy. Anthropometric measurements were obtained on all participants. Participants were classified into four groups: with and without central obesity and reflux.

Results: Sixteen individuals were studied with four in each study group. The mean ICSD was almost three-fold greater ($p < 0.001$) in the group with central obesity without reflux, compared to controls without central obesity and reflux. It was also comparable to the ICSD in groups with acid reflux only and those with both reflux and central obesity.

Conclusions: There is evidence of esophageal squamous ICSD increase in individuals with central obesity who do not have evidence of acid and nonacid reflux on ambulatory pH monitoring. This may reflect a mechanism by which central obesity potentiates reflux-induced esophageal injury and inflammation.

Keywords

Central obesity, intercellular dilation, electron microscopy, Barrett's, esophageal reflux

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Introduction

The incidence of esophageal adenocarcinoma (EAC) and obesity are increasing rapidly.¹ Central obesity is an independent risk factor for Barrett's esophagus (BE) and EAC.² Central obesity is associated with mechanical disruption of the gastroesophageal junction (GEJ) and increased gastroesophageal reflux (GER) that causes esophageal injury.^{3,4} In addition, central obesity (and visceral abdominal fat) has been shown to be a reflux-independent risk factor for esophagitis and BE, suggesting a non-mechanical mechanism of action.^{3,5} However, the potential mechanisms by which visceral abdominal fat released cytokines and adipokines predispose to esophageal mucosal injury are unknown.

Obesity has been implicated to increase intestinal permeability in animal models.^{6–9} Epithelial tight junctions that maintain epithelial integrity can be damaged by circulating proinflammatory cytokines released from visceral abdominal fat in centrally obese individuals.^{10,11} In addition, GER has been shown to lead to esophageal epithelial barrier damage characterized

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by dilated intercellular spaces (DIS) in the squamous epithelium.¹²

Impairment of the epithelial barrier in central obesity could facilitate paracellular permeation of noxious compounds in the refluxate, accentuating the injury and inflammation cascade, and promoting esophageal metaplasia and neoplasia. This phenomenon could provide the “second hit” for the development of BE in a background of mildly increased or physiologic levels of GER.¹³ There are currently limited data on the morphological characterization of the esophageal epithelial barrier in individuals with increased abdominal visceral fat (with and without reflux). In addition it is also unclear if the effects of these two risk factors are additive when present concurrently.

We hypothesized that the esophageal epithelial barrier (as determined by the squamous epithelial intercellular space diameter (ICSD)) is altered in patients with central adiposity without GER. The aim of this study was to: 1) determine the independent effects of central obesity and GER on the intercellular space diameter (ICSD) in the esophageal squamous epithelium of individuals with central obesity with and without physiologic evidence of GER; and 2) determine the effect of these risk factors on the ICSD when present in isolation and in combination.

Methods

This was a prospective cohort study. Sixteen individuals who underwent clinically indicated ambulatory esophageal pH monitoring were recruited to this study. Ambulatory pH monitoring was performed using a 24-hour combined pH impedance catheter assembly (Given, Yoqneam, Israel) with the proximal pH sensor placed at 5 cm from the upper border of the manometrically localized lower esophageal sphincter or a 48-hour wireless pH recording using the radiocapsule (Bravo) catheter-less technique (Given, Yoqneam, Israel). None of these patients had endoscopic evidence of BE (columnar mucosa in the distal esophagus >1 cm in length), a history of prior esophageal/gastric surgery or prior chemotherapy or radiation. Anthropometric measurements (height, weight, waist and hip circumference) were obtained using standard methods by a trained research coordinator. All participants underwent upper endoscopy 24–48 hours after completing the ambulatory pH study. Research biopsies were taken from the esophageal squamous mucosa at 5 cm above the GEJ following patient consent. The tissue was then formalin fixed, paraffin embedded, cut into 1 μ thick sections and stained with toluidine blue to assist in selecting the appropriate area to make the ultrathin sections (Figure 1).

Sections 60 nm thick were then prepared from the squamous mucosal biopsies. Using a JEOL ExII Transmission Electron Microscope, four images at 4000 \times power were obtained randomly at the prickle layers in the Optical Microscopy Core Laboratory (Figure 1). Then, using a computerized image analyzer (Digimizer, version 4.2.2.0, MedCalc Software), 10 transects were randomly drawn across perpendicular cell membranes with each transect no closer than 1 μ m apart. The intercellular space was calculated at these transecting lines using standard and commonly accepted techniques by an observer who was blinded to the clinical features.^{12,14} The measurements were obtained at the prickle layer of each patient to maintain consistency between patients, given the known inter-layer variability that has been reported.¹⁵ The mean ICS measurement for each patient was calculated from the four sets of 10 measurements taken in the photomicrographs.

All pH/impedance studies were read by a single investigator (PGI) who was blinded to other clinical information. Excessive total distal esophageal acid exposure was defined as greater than 4.2% of the time with a pH less than 4 in the distal esophagus over 24 hours and a composite DeMeester score of >14.7. For those who underwent impedance pH testing, we defined an abnormal number of total reflux episodes to be >48 in those on proton pump inhibitors (PPIs) and >73 in those off PPIs.¹⁶ The number of total, acid and nonacid reflux episodes were also separately assessed in those undergoing pH-impedance testing on PPIs (11 out of 16 participants) and were also used for classifying individuals into the study groups defined below.

Patients were divided into four groups: Group 1: Normal body mass index (BMI) (19–25 kg/m²) and waist-to-hip ratio (WHR) (women < 0.85 and men < 0.90), physiologic number of acid and nonacid reflux events (if on PPI); Group 2: Increased BMI (>30 kg/m²) and WHR (women > 0.85 and men > 0.90) and physiologic number of acid and nonacid reflux events (if on PPI); Group 3: Normal BMI and WHR with pathologic GER (greater than 4.2% time with pH less than 4 or elevated total number of reflux events (if on PPI); Group 4: Increased BMI and WHR with pathologic GER (greater than 4.2% time with pH less than 4 or elevated total number of reflux events (if on PPI). The WHR cutoff used for defining central obesity was as per World Health Organization standards.¹⁷

Statistical analysis was conducted using JMP[®] 10.0.0 statistical software (SAS Institute). Data were summarized as the mean (standard deviation) or median (interquartile range) as per data distribution. Comparison between groups was performed using the analysis of

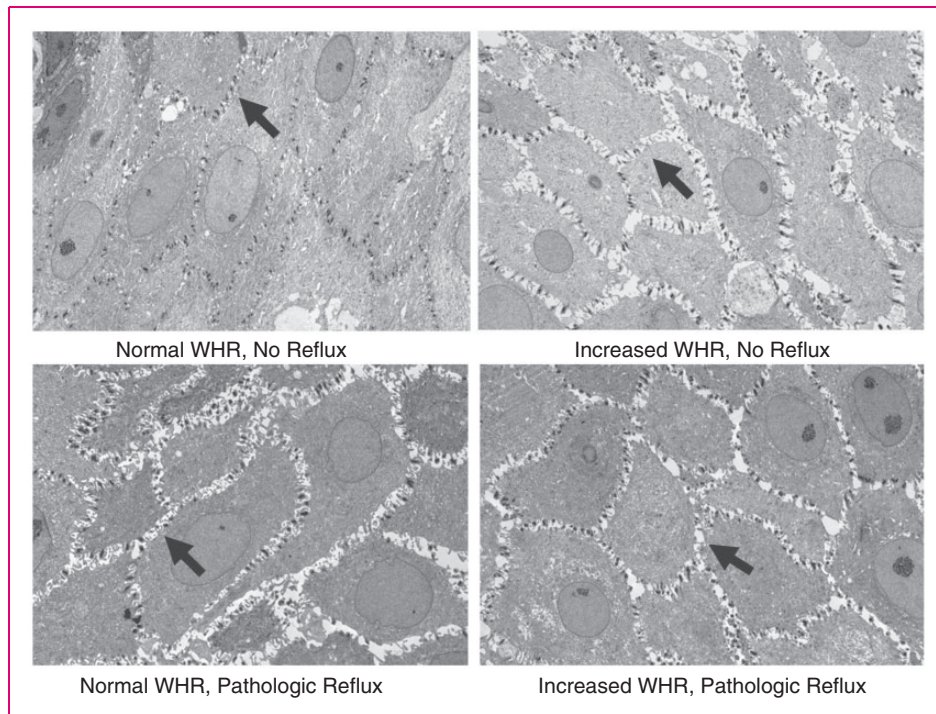


Figure 1. Representative photomicrographs of intercellular spaces in the squamous esophageal epithelium of individuals with and without reflux and central obesity (measured using waist-to-hip ratio (WHR) as defined in the manuscript (4000 \times).

Table 1. Demographic and clinical features of patients in the study as divided into four predetermined study groups

	Group 1 (N=4)	Group 2 (N=4)	Group 3 (N=4)	Group 4 (N=4)	p value
Mean age (SD), years	47.3 (23.5)	49.8 (19)	36.8 (9.8)	35.8 (28.1)	0.72
Male gender (%)	50	25	25	0	0.89
Mean BMI (SD)	22.5 (1.2)	35.4 (2.9)	23.1 (1.8)	37.5 (3.5)	<0.0001
Mean WHR (SD)	0.79 (0.03)	0.82 (0.06)	0.97 (0.04)	0.96 (0.09)	0.002
Mean % time pH <4 in distal esophagus (SD)	1.4 (1.9)	1.1 (1.1)	5.8 (2.1)	10.3 (6.9)	0.015
Mean number of total reflux episodes (N, SD) ^a	39 (4, 26.3)	42.3 (4, 13.7)	60.5 (4, 32.4)	105 (4, 25.6)	0.012
Mean number of nonacid reflux episodes (N, SD)	11 (2, 11.3)	31.3 (4, 5.3)	28 (4, 9.3)	91 (1, -)	0.001
Symptomatic reflux as indication of EGD (%)	75	25	75	100	0.137
Smoking (%)	0	0	25	0	0.362
Alcohol consumption (%)	50	50	50	75	0.859
PPI or H2 antagonist use (%)	50	50	75	100	0.362
NSAID or ASA use (%)	25	50	0	50	0.362

^aIncludes patients with 48-hour Bravo study or 24-hour impedance study.

BMI: body mass index; WHR: waist-to-hip ratio; EGD: esophagogastroduodenoscopy; PPI: proton pump inhibitor; NSAID: nonsteroidal anti-inflammatory drug; ASA: acetylsalicylic acid.

variance (ANOVA) (for continuous variables) or the chi square test (for dichotomous variables).

Results

Demographic and clinical features of the 16 patients who underwent ambulatory pH monitoring, endoscopy

with research biopsy and anthropometric measurements and were divided into the four study groups described above are shown in Table 1. Age, smoking, alcohol consumption, and medication use were not significantly different among groups ($p > 0.05$). The ratio of men to women was higher in Group 1. Indications for endoscopy, as documented in a clinic

visit with a gastroenterologist, included esophageal (heartburn, regurgitation) and extra-esophageal (cough, hoarseness) reflux symptoms (10, 63%), dysphagia (two, 12.5%), nausea/vomiting (one, 6%), dyspepsia (one, 6%), non-cardiac chest pain (one, 6%), and asthma (one, 6%).

Eleven (69%) patients were on PPIs at the time of ambulatory pH testing, of whom one patient was on a histamine receptor antagonist as well at the time of pH testing. Five of 16 participants underwent ambulatory pH monitoring using the Bravo pH monitoring system (performed off medication) with the remaining undergoing combined pH impedance testing (performed on medication). Of the two patients in Group 1 who were on a PPI, the one who completed the Bravo study had an esophageal pH that was <4 for 0.8% of the recording time and the other patient who underwent 24-hour impedance had only three episodes of acid reflux with the pH being <4 for 0.4% of the time. This is far below the 95th percentile of the number of GER episodes in those on PPI in previous studies.¹⁸

As seen in Table 1, the mean percentage time pH was less than 4 in the distal esophagus was substantially lower and in the physiologic range in Groups 1 (no central obesity and no reflux) and 2 (centrally obese and no reflux) and elevated in Groups 3

(no central obesity, with reflux) and 4 (centrally obese with reflux). The mean number of nonacid reflux events in participants in Groups 1, 2 and 3 who underwent pH impedance testing (two individuals in Group 1: 11 events, four patients in Group 2: 31 events and four patients in Group 3: four events) was also in the physiologic range,¹⁸ with only one patient in Group 4 (centrally obese, with reflux) demonstrating an elevated number (91) of nonacid reflux episodes. This suggests that participants in Groups 1, 2 and 3 did not have evidence of excessive nonacid reflux as well.

At endoscopy, the esophageal mucosa was normal in 13 (81%) patients. Two patients (13%) had esophagitis, one each with Los Angeles classification-A (Group 4) and Los Angeles classification-B (both in Group 3), with increased GER on pH monitoring. One patient (6%) with treated eosinophilic esophagitis had esophageal furrowing and a normal esophageal pH study. Biopsies revealed histologically quiescent disease.

As seen in Figures 1 and 2, patients in Group 1 (i.e. controls with no reflux and no central obesity) had the smallest ICSD, with a mean (SD) ICSD of $0.275 \mu\text{m}$ (0.1). The ICSD was more than twofold greater in Groups 2, 3 and 4 than Group 1; each of these differences was statistically significant ($p < 0.0001$). The mean (SD) ICSD for these groups were $0.753 \mu\text{m}$ (0.4), $0.826 \mu\text{m}$ (0.3) and

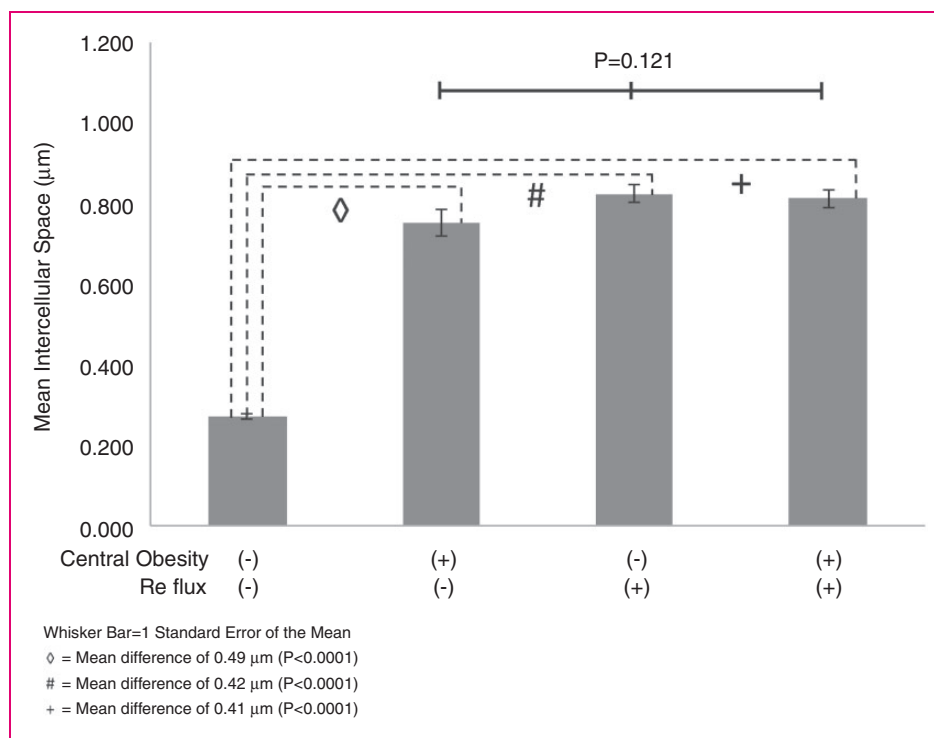


Figure 2. Comparison of intercellular space diameter measurements from the esophageal squamous epithelium of groups with and without central obesity and reflux.

Table 2. Influence of clinical variables on the intercellular space diameter in the esophageal squamous epithelium on transmission electronic microscopy in the four study groups

	Men	Women	<i>p</i> value
Sex	0.4 (4, 0.23)	0.76 (12, 0.25)	0.03
	Yes	No	
Tobacco use	0.99 (1, -)	0.65 (15, 0.28)	0.25
Alcohol use	0.64 (9, 0.31)	0.69 (7, 0.28)	0.727
Aspirin use	0.61 (4, 0.36)	0.69 (12, 0.28)	0.69
NSAID use	0.64 (3, 0.33)	0.67 (13, 0.29)	0.87
PPI or H2 antagonist use	0.69 (11, 0.28)	0.62 (5, 0.34)	0.685

Key: Mean in (N, SD)

NSAID: nonsteroidal anti-inflammatory drug; PPI: proton pump inhibitor.

0.814 μm (0.3), respectively. However, differences between the ICSD of Groups 2, 3 and 4 were not significant ($p = 0.12$). Increased esophageal ICSD was not associated with age, tobacco use, alcohol use, PPI or H2 receptor antagonist (H2RA) use (Table 2). The mean ICSD was higher in females than males. There was no statistical difference in ICSD between those on an acid-suppressing medication versus those who were not.

Discussion

Dilation of intercellular spaces in the esophageal squamous epithelium has been shown to be an early pathologic event in GER disease (GERD). In this small but prospective study with four carefully characterized patient groups with and without central obesity and GER (both documented using validated objective criteria), we have demonstrated that this pathologic change occurs not only in response to abnormal esophageal acid exposure (Groups 3 and 4) but also in patients with central obesity without evidence of acid or nonacid reflux on ambulatory pH monitoring (Group 2). Specifically, our data show that among patients with central obesity with or without GER (Groups 2, 3), the ICSD of the squamous esophageal epithelium was significantly greater than in controls without central obesity or reflux (Group 1). Furthermore, the ICSD in patients with central obesity with or without GERD was comparable to that of patients with GER alone in this and previous studies of patients with GERD.¹² Our results were not affected by relevant clinical variables.

This study supports two important concepts in GERD. The first is that dilation of intercellular spaces in esophageal epithelium is an early event in and sensitive marker for GERD as has been shown

in previous studies.^{8,9} Moreover, reflux did not augment the effect of obesity on ICSD dilation, suggesting a threshold effect attributable to obesity. From a mechanistic view, these early changes makes intuitive sense as the dilation of these spaces allows diffusion of HCl-rich fluid into the esophageal epithelium thereby facilitating caustic injury and potential cytokine release.¹⁹ The pathogenesis of this process is unclear but, conceivably, disruption of the structure or function of tight junction proteins may, at least partly, mediate these effects.²⁰ The mechanism by which acid induces these changes is unclear and warrants further study. In addition, the role of nonacid reflux on intercellular space dilation is unclear. Of the 11 patients in our study who underwent impedance testing, the mean number of nonacid reflux episodes was 33, which is well below the 73 episodes that is thought to be pathologic.¹⁶ The low rate of nonacid reflux in our study patients likely mitigates the potential confounding nature of nonacid reflux on ICSD dilation.

The second important concept is that central obesity is associated with a morphologically impaired esophageal epithelial barrier in the absence of pathologic levels of acid exposure. Since central obesity may predispose to GER by mechanically disrupting the GEJ reflux barrier and increasing intra-abdominal pressure, it also makes intuitive sense that obesity leads to dilation of intercellular spaces by promoting GER. Consistent epidemiologic evidence, however, suggests a reflux-independent mechanism by which central obesity may increase the risk of esophageal inflammation and neoplasia.²¹ Indeed, with stratification of groups by acid reflux and central obesity status that enabled an accurate and objective assessment of the effects of central obesity independent of acid reflux, we demonstrated that ICSD occurs in centrally obese patients in the absence of documented GER. Thus, disruption of the esophageal epithelial barrier may be an underlying mechanism by which increased visceral abdominal fat increases the susceptibility of the esophageal epithelium to reflux-mediated injury and inflammation that potentiates the development of metaplasia and neoplasia.³

The cause of ICSD without pathologic reflux is unclear but it is postulated that the disruption of tight junctions may occur through pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF α) and interleukin 6 (IL6), which are elevated in obese patients. It has also been demonstrated that TNF α decreases barrier function in esophageal epithelium.²² Indeed, these cytokines have been shown to be involved in remodeling tight junctions by altering epithelial barrier proteins such as claudins and occludins.²³ Notably, recent data from our group in patients with eosinophilic esophagitis, a strongly cytokine-mediated

disease, also demonstrate that pathologic dilation of intercellular spaces correlates inversely to tight junction protein expression.²⁴

This study has some potential limitations. We recognize the small sample size of this study and conducted this exploratory study to obtain pilot data, but were struck by the statistically significant differences between Group 3 (centrally obese with evidence of excessive acid or nonacid reflux) and the control groups (Group 1 and 3). These data should be confirmed in larger studies. While most participants were on PPIs, the lack of not only excessive acid reflux but also total and nonacid reflux events (particularly in those on PPIs in Groups 1 and 2) argues against misclassification bias. Moreover, the mean ICSD values in Groups 1 (normal controls) and 3 (excessive acid and nonacid reflux) are comparable to those described in the literature as well. It is conceivable that the increased ICSD in centrally obese individuals without pathological GER (i.e. Group 2) reflects the effects of ongoing but undetected GER, i.e. the pH study was falsely negative. However, this seems unlikely given the extremely low mean acid exposure duration in this group, which was lower than in Group 1, and normal findings on endoscopy. Furthermore, extra-esophageal symptoms of GERD were the predominant indication for pH testing in Group 2 (with central obesity, without reflux). We cannot exclude the possibility that the increased ICSD in Group 2 is attributable to weakly or nonacidic and/or bile reflux, which has been reported in animals.²⁵ The physiologic number of nonacid reflux episodes in patients in Group 2, however, makes this relatively unlikely. Lastly, there are various methods for assessing epithelial ICSD that include routine histologic analysis assessing spongiosis.²⁴ We chose electron microscopy as this appears to be the most objective and precise method. We also ensured that all our photomicrographs included the basal membrane for orientation, which is critical for obtaining standardized measurements.¹⁵

In summary, central obesity with or without reflux may be associated with DIS, reflecting morphological impairment of the esophageal epithelial barrier. These observations should be confirmed in larger studies. This early change, which does not appear to be additive in the presence of concomitant reflux, may occur independently of either central obesity or gastroesophageal reflux. Further studies are necessary to clarify if the pathogenesis of DIS involves cytokine-mediated or independent mechanisms.

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Author contributions are as follows:

Christopher H Blevins was responsible for the study concept, study design, data acquisition, data analysis, drafting the manuscript and statistical analysis.

Anamay N Sharma was responsible for technical and material support.

Michele L Johnson was responsible for administrative, technical and material support.

Deborah Geno was responsible for administrative, technical and material support.

Milli Gupta was responsible for acquisition of data and study concept.

David A Katzka was responsible for critical review of manuscript for important intellectual content.

Adil E Bharucha was responsible for critical review of manuscript for important intellectual content.

Prasad G Iyer was responsible for the study concept, study design, critical revision of the manuscript for important intellectual content, obtained funding support and provided study supervision.

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Conflict of interest

None declared.

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