Annals of Gastroenterology (2023) **36,** 504-510

The brain–esophagus axis in subjects with and without obesity assessed by esophageal acid perfusion and functional brain imaging

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Abstract

Background Gastroesophageal reflux disease (GERD) has a complex pathophysiology and a heterogeneous symptom profile. The brain–esophageal axis in GERD has been studied with functional brain imaging during the last decades, but data from obese patients was just recently reported. A comparison of such a group with non-obese subjects is lacking in the literature. This study aimed to evaluate heartburn perception and brain connectivity responses during esophageal acid stimulation in subjects with and without obesity, controlling for the presence of typical reflux symptoms.

Methods In this cross-sectional study, 25 patients with obesity (body mass index ≥30 kg/m^2) and 46 subjects without obesity underwent functional magnetic resonance imaging (fMRI) of the brain with esophageal water and acid perfusion. The fMRI paradigm and connectivity were assessed.

Results About two-thirds of the participants had reflux symptoms. Heartburn perception during fMRI did not differ between subjects with and without obesity. The presence of reflux symptoms was associated with lower activation in frontal brain regions during acid perfusion compared to water perfusion. Compared to subjects without obesity, patients with obesity presented significantly lower connectivity within the anterior salience network. Corrected clusters included left caudate, left putamen and left anterior cingulate gyrus.

Conclusions The brain–esophagus axis showed differences between subjects with and without obesity. Even without symptomatic differences following esophageal acid perfusion, patients with reflux symptoms showed less brain activation in frontal areas, while obese individuals presented lower connectivity within the anterior salience network.

Keywords Functional magnetic resonance imaging, brain activation, heartburn perception, gastroesophageal reflux disease

Ann Gastroenterol 2023; 36 (5): 504-510

Conflict of Interest: None

Funding: This study was partially supported by Clínica Kozma and Endopasso

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Received 5 February 2023; accepted 24 May 2023; published online 3 July 2023

DOI: https://doi.org/10.20524/aog.2023.0818

Introduction

Gastroesophageal reflux disease (GERD) is a relevant condition with a high prevalence, complex pathophysiology and a heterogeneous symptom profile [1]. Its modern diagnosis relies on the application of consensual parameters on esophageal testing for conclusive identification of GERD phenotypes [2]. The prevalence of GERD is higher in the population with obesity [3]. This results from conditions related to obesity, including eating behaviors, higher gastric pressure, visceral fat-related inflammation [4] and proximal dislocation of the gastric pocket [5]. In addition, hiatal hernia and transient lower esophageal sphincter relaxations are more frequent in obese patients [6,7].

Esophageal sensitivity to reflux of gastric contents is based on the activation of chemo- and mechanoreceptors [8], with variations according to GERD phenotypes. Overall, patients with functional heartburn show higher sensitivity to chemical and mechanical stimuli than other GERD phenotypes [9]. In obese individuals, esophageal mechanisms such as epithelial resistance and visceral sensitivity are less understood [10]. Although most studies have described esophageal hyposensitivity with high preoperative rates of reflux esophagitis in oligosymptomatic candidates for bariatric surgery [11,12], there is some evidence suggesting a visceral hypersensitivity associated with obesity [13].

The application of validated questionnaires for GERD identification is widely accepted in clinical research, despite their acknowledged flaws [14]. Such instruments can translate the subjectivity of GERD manifestations into objective symptom scores, guiding both diagnosis and responses to therapeutical strategies. However, GERD questionnaires have limited accuracy when compared to objective reflux testing [2], with sensitivity and specificity inferior to 70%.

New technologies have enabled the evaluation of brain responses related to visceral stimulation [15]. Functional magnetic resonance imaging (fMRI) has been used to assess the brain–esophagus axis and the relationship with perception of heartburn in volunteers and patients with GERD [16,17]. This tool allows both task analysis as a response to esophageal acid stimulation and the assessment of task-residual connectivity [18]. Studies with this technology, combined with esophageal acid perfusion, have described different brain activation patterns among the GERD subtypes [19].

The evaluation of the brain–esophageal axis was recently characterized in patients with obesity [20,21]. We aimed to assess heartburn perception and brain connectivity responses during esophageal acid stimulation in subjects with and without obesity, controlling for the presence of reflux symptoms.

Patients and methods

Participants

In this cross-sectional study, which included a retrospective analysis of a prospectively built database, 26 patients with obesity (body mass index [BMI] ≥30 kg/m^2) and 46 subjects without obesity were recruited and studied from 2010-2015, following a unique protocol for data acquisition [22]. Among the obese subjects there were 9 healthy volunteers, 15 patients with troublesome heartburn and normal esophageal mucosa on endoscopy, and 2 patients with troublesome heartburn and erosive esophagitis. The non-obese subjects included 17 healthy volunteers, 18 patients with troublesome heartburn and normal esophageal mucosa on endoscopy, and 11 patients with troublesome heartburn and erosive esophagitis.

The inclusion criteria for all participants were: (1) older than 18 years; (2) right-handed; and (3) consent to participate. Exclusion criteria were: (1) uncompensated clinical morbidities; (2) gastroesophageal surgery; (3) claustrophobia; (4) regular use of acid-suppressive medications or drugs acting on the central nervous system; (5) pregnancy; and (6) use of metal orthodontic appliances. Reflux symptoms were characterized in the presence of troublesome heartburn and/or acid regurgitation evaluated with a validated GERD symptoms questionnaire [23]. All participants apart from the healthy volunteers without obesity underwent upper digestive endoscopy prospectively or in the last 6 months from date eligibility. Reflux esophagitis was identified according to Los Angeles classification (grades B, C and D). Anxiety and depression scores were obtained with the Hamilton Anxiety Scale and Beck Depression Inventory version 1A, respectively [24,25]. All participants gave their informed consent and the study was approved (numbers 150/2009 and 383/2011) by the local Ethics Committee.

Study protocol

Esophageal acid stimulation

A mono-lumen catheter was inserted trans-nasally and positioned at 5 cm proximally to the lower esophageal sphincter according to a previously mapped pH turning point, as described elsewhere [26]. After approximately 15 min from catheter placement, the patients were placed in the fMRI machine. Subjects were instructed to lie still during the exam and signal with one finger of the left hand when heartburn was perceived. The experimental protocol included an anatomical scan followed by 2 periods of 5 min each, with esophageal perfusion at 1 mL/min. The first perfusion was performed with distilled water (pH 6.5) and the second with a solution based on hydrochloric acid (pH 1.5). The acid solution was prepared by a compounding pharmacy, with hydrochloric acid added to distilled water until its pH stabilized at 1.5. In both perfusion periods, we acquired functional images of the brain. Although the perfusion order was not randomized, the patients were blinded to the solution type sequence.

fMRI data acquisition

Magnetic resonance images were acquired at Clínica Kozma (Passo Fundo, RS, Brazil) using a 1.5 Tesla Magnetom Avanto (Siemens AG, Munich, Germany), equipped with a standard 8-channel head coil. High-resolution tridimensional anatomic images were obtained from 176 sagittal spoiled gradient recalled sequence slices with a voxel size of 1.0 × 1.0 × 1.0 mm,

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for further co-registration with functional images. For functional acquisition, echo planar images were obtained in a mosaic composed by 36 axial slices of 64 × 64 pixels over a 220 mm field of view, with a voxel size of 3.0 × 3.0 × 3.75 mm, a repetition time of 4270 msec and an echo time of 50 msec.

MRI preprocessing and first-level analysis

MRI functional images were preprocessed using the FMRIB Software Library (FSL) [27]. Brain extraction was performed using the Brain Extraction Tool [28]. Functional data preprocessing was then carried out using the FEAT program standard pipeline for first-level analysis [29]. Data underwent motion correction, slice timing correction and Gaussian kernel smoothing full width at half maximum of 4.0 mm. Functional data were co-registered with a subject-respective T1 anatomical image. Anatomical data were registered in standard MNI152 space, using FLIRT with 12 degrees of freedom, and the resulting transformation matrix was applied to warp co-registered functional data into standardized space. We then, modeled a task time series within FEAT [30], modeling the hemodynamic response function assuming a sinusoidal function for water and acid perfusion effects on signal variance.

Task-residual functional connectivity (trFC) analysis

After first-level analysis, the resulting residuals (effects not explained by task), underwent independent component analysis (ICA) carried out with MELODIC [18]. ICA analysis decomposed each participant's functional data into spatial and temporal components, and concatenated all subjects' components using multi-session temporal concatenation to generate group-spatial maps. Non noise-related components of interest were selected according to a functional network atlas [31]. From the group-selected components we created masks using a threshold of z>2.3 that would be utilized further in group comparisons.

Statistical analysis

Group comparisons between subjects for task data were made using the FLAME1 part of FEAT for higher-level analysis [32], which uses mixed effects modeling to estimate signal parameters. We account for normalized BMI as a continuous variable and the presence of reflux symptoms as a dummy variable in our task model; cluster correction for multiple comparisons was than performed, using a threshold of z>2.3. trFC group comparisons were carried out in FSL using dual regression and the program Randomise. First, group-spatial-maps were regressed for each specific subject time series, after those time series had been regressed into the same 4D data to generate subject-specific spatial maps. Eventually, Randomise was used to compare groups. We accounted for normalized BMI as a continuous variable and reflux symptoms status as a dummy variable in the first model, and in the second model, we accounted for obesity (BMI >30 kg/m^2) as a dummy variable (2-sample independent *t*-test). Randomise was carried out using 5000 permutations, and cluster correction for multiple comparisons; a P-value <0.05 for corrected clusters was considered significant. From significant clusters, we created region of interest (ROI) masks and warped them to subject-specific native space, to extract connectivity z-score values from those ROIs. Demographic and clinical characteristics were compared between obese and non-obese using the Student's *t*- and χ-square tests for quantitative and qualitative variables, respectively. In the case of asymmetric quantitative data according to the Kolmogorov-Smirnov test, the Mann-Whitney *U* test was used. Analyses were performed with GraphPad Prism version 5 (GraphPad Software Inc, San Diego CA, USA) and Statistical Package for Social Sciences version 16.0 (SPSS Inc, Chicago, IL, USA). The P-value defined as significant was 0.05.

Results

Participants

A total of 72 subjects were enrolled in the study. One participant was excluded because of an artifact on the fMRI data. The 71 remaining participants included 25 obese patients and 46 without obesity (Table 1). Women and men were equally distributed in both groups, whereas participants with obesity were slightly older. The morbidity variables, including reflux symptoms, depression and anxiety, were all similar between groups. Troublesome reflux symptoms and/or reflux esophagitis were found in approximately 65% of the participants in both groups.

Heartburn perception during fMRI

The perception of heartburn did not differ between subjects with and without obesity, being signaled by approximately 40% of the participants (Table 2). A subgroup analysis of participants with reflux symptoms (n=46) also did not show differences in heartburn perception between groups. Among 6 participants with obesity in which the heartburn signaling was discriminated according to perfusion type, 24% of heartburn episodes were signaled during water perfusion and 76% during acid perfusion in the esophagus.

Brain responses to esophageal acid stimulation

Task activation

The presence of reflux symptoms was associated with lower brain activation in frontal brain areas during acid perfusion compared to water perfusion, after controlling for BMI

Table 1 Characteristics of the participants (n = 71)

Characteristics	Obese n = 25	Non-obese n = 46	P-value
Women, n (%)	11 (44)	26 (56)	0.333
Age in years, mean ± SD	39.4 ± 9.9	33.6 ± 11	0.032
BMI in kg/m², mean ± SD	33.5 ± 3.8	24.3 ± 3.2	<0.001
Reflux symptoms/RE*, n (%)	17 (68)	29 (63)	0.797
Symptom questionnaire, median (IQR**)	6 (5-16)	8 (0-13.3)	0.425
Symptom questionnaire>10 points, n (%)	10 (40)	19 (41)	0.999
RE†, n/performed endoscopy (%)	9/25 (36)	11/29 (38)	0.999
Depression			
Median score (IQR)	6 (3-10.5)	4 (1-10)	0.171
Anxiety			
Median score (IQR)	8 (3-15)	7 (3-13.7)	0.965

*Troublesome typical symptoms and/or RE (Los Angeles B, C and D) **IQR 25-75%
BMI, body mass index; SD, standard deviation; RE, reflux esophagitis; IQR, interquartile range

Table 2 Heartburn perception (and score) during fMRI with esophageal perfusion (5 min water and 5 min acid) in subjects with and without obesity (all participants, regardless of reflux symptoms/RE), and in obese and non-obese patients with reflux symptoms/RE (Los Angeles B, C or D)

Parameters	Obese	Non-obese	P-value
All participants (n = 71)	n = 25	n = 46	
Heartburn yes/no (%)	9/16 (36)	19/27 (41)	0.800
Heartburn score, median (IQR*)	0 (0-2.5)	0 (0-3.5)	0.902
Reflux symptoms/RE (n = 46)	n = 17	n = 29	
Heartburn yes/no (%)	8/9 (47)	15/14 (52)	0.999
Heartburn score, median (IQR*)	0 (0-7.5)	1 (0-5.5)	0.980

*IQR 25-75%
RE, reflux esophagitis; IQR, interquartile range; fMRI, functional magnetic resonance imaging

Table 3 Significant activation clusters, associated with the presence of typical reflux symptoms (control > presence of symptoms), comparing acid perfusion with water perfusion

Brain region*	Voxel size	Max z-stat	Max X	Max Y	Max Z
Left frontal orbital cortex	65	4.19	66	80	27
Left frontal pole	55	4.09	67	90	29
Right frontal orbital cortex	35	4.58	28	78	27
Right frontal inferior gyrus	21	3.85	25	81	32

*In participants with reflux symptoms, esophageal acid perfusion was associated with lower activation in frontal brain areas, compared to water perfusion

(Table 3). Significant clusters included left and right frontal orbital cortex, right inferior frontal gyrus and left frontal pole. BMI was not associated with significant changes in brain activation, after adjustment for reflux symptom status.

trFC

In the model using BMI adjusted for reflux symptom status, no significant changes were found in any functional network. In the model with dichotomous BMI (obese vs. nonobese), patients with obesity presented significantly lower connectivity within the anterior salience network (Fig. 1, 2), in the left caudate, left putamen and left anterior cingulate gyrus regions. Cluster information with mean normalized connectivity for each group is shown in Table 4. There were no significant differences between groups within the default mode network, sensorimotor network, executive network or primary visual network.

Discussion

In this study involving participants with and without obesity, with a variety of GERD phenotypes, we investigated whether heartburn perception and brain responses during esophageal acid stimulation were different in relation to

Table 4 Significant trFC clusters, associated with obesity status within the anterior salience network (non-obese>obese)

Brain Region	Voxel size	Max P-value	Max X	Max Y	Max Z	Mean obese connectivity (SD)	Mean non-obese connectivity (SD)
Left caudate and left putamen	106	0.019	55	73	35	0.89 (0.98)	2.4 (1.3)
Left anterior cingulate gyrus	37	0.038	47	79	42	1.59 (0.9)	3.24 (1.7)

SD, standard deviation; trFC, task-residual functional connectivity

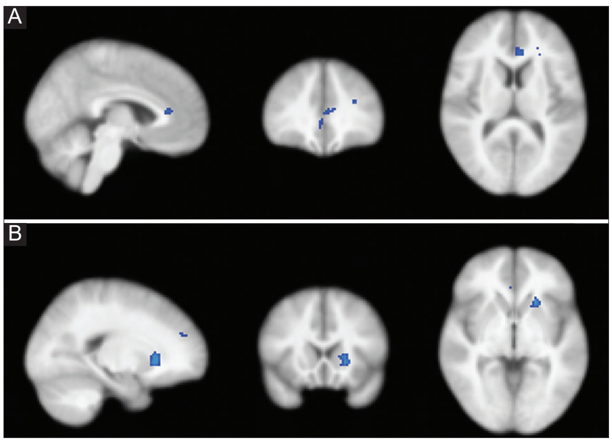

Figure 1 Differences in task-residual functional connectivity between subjects with and without obesity within the anterior salience network. (A) Left anterior cingulate gyrus cluster. (B) Putamen and caudate cluster. Blue codifies lower values in patients with obesity

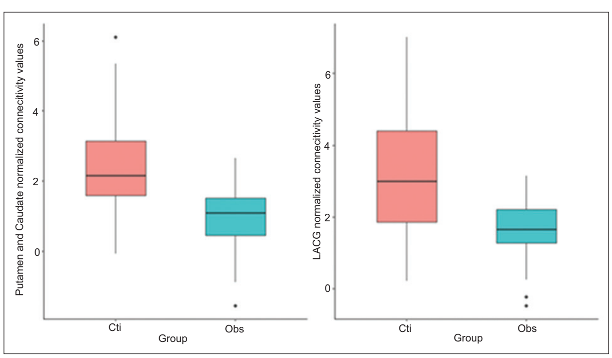

Figure 2 Mean connectivity values within the anterior salience network for each group in each cluster. Left image shows mean connectivity in the putamen and caudate nuclei. Right image shows mean connectivity for the left anterior cingulate gyrus (LACG). Patients with obesity correspond to the blue boxplot and controls to the orange

obesity. Both task analysis, as a response to esophageal acid stimulation, and task-residual connectivity were obtained with fMRI. As far as we know, this is the first study addressing the brain–esophagus axis in subjects with and without obesity in the context of reflux symptoms.

The main findings were: (a) overall perception of heartburn during esophageal acid perfusion was similar between groups; (b) among patients with reflux symptoms, the perception of heartburn also did not differ, regardless of obesity; (c) compared to controls, patients with obesity presented significantly lower connectivity within the anterior salience network, including the left caudate, left putamen and left anterior cingulate gyrus; (d) patients with reflux symptoms presented lower brain activation related to esophageal acid perfusion in frontal brain regions; and (e) participants with and without obesity were balanced in terms of depression and anxiety scores, softening the influence of these emotional conditions on results.

Visceral hypersensitivity is more commonly reported in patients with functional gastrointestinal diseases, such as functional heartburn [33]. This mechanism, represented by the brain–esophagus axis, contributes to functional esophageal pain and consists of a complex interaction between peripheral nerves, superior cortical centers, a descending inhibitory pathway and psychological modulation triggered by the stimulus [34]. Few studies have addressed the role of obesity in esophageal sensitivity in the context of GERD. Mercer *et al* compared asymptomatic subjects with obesity and lean volunteers and found esophageal hypersensitivity to acid perfusion in the group with obesity [13]. In contrast, Ortiz *et al* reported that asymptomatic GERD is more common than the symptomatic pattern in patients with severe obesity (BMI ≥40 kg/m^2) [11].

In the present study, the perception of heartburn during esophageal acid stimulation was similar in both groups. Acid perfusion in the distal esophagus lasted 5 min using a solution with very low pH (~1.5) and was able to trigger heartburn in approximately 40% of the participants. This model of esophageal stimulation followed the patterns proposed by Kern *et al*, pioneers in the evaluation of the brain–esophageal axis in patients with GERD [16]. The use of a multimodal esophageal stimulation paradigm [35], including thermal, mechanical, electrical and chemical (acidic solution) stimulations, could provide additional data regarding esophageal sensitivity.

The limbic system, which includes the insular, cingulate, amygdala, thalamus, hypothalamus and prefrontal cortex, was reported to participate in the processing of afferent sensory inputs of the esophagus [16,36]. In particular, the insula and cingulate play a role in the integration of autonomic, affective, cognitive and motor responses to sensory signals originating from the gastrointestinal tract [37]. The prefrontal lobe cortex is the senior center of algesthesia. Somatic and visceral sensory information converge on the insula, which can manage sensation and emotion. In fact, studies comparing GERD patients with controls have demonstrated abnormal default mode network and insular cortex functional connectivity in the patients, reinforcing the role of such areas in the esophageal perception of reflux [38,39]. However, none of these studies specifically examined the influence of obesity on the results.

In our study, patients with obesity presented significantly lower connectivity within the anterior salience network, including the left caudate, left putamen and left anterior cingulate gyrus, compared to subjects without obesity. This finding may be an initial explanation for asymptomatic GERD being more common than the symptomatic pattern in those with severe obesity [11]. On the other hand, lower connectivity in obese subjects was already reported during the resting state or milkshake consumption, suggesting that this finding may be a non-specific response due to obesity [40,41]. The anterior salience network is part of the "default mode of brain function", defined as a task-negative network, the activity of which is greater at rest than under various goal-directed tasks [42]. In addition, participants with reflux symptoms presented lower brain activation related to acid perfusion in frontal brain regions compared to participants without reflux symptoms. However, in the model using BMI adjusted for reflux symptom status, no significant change was found in functional networks.

Most neuroimaging studies of the brain–esophageal axis are based on intermittent or continuous esophageal stimulation. Though these are not stimulus-specific, anticipation of stimuli can trigger similar activity, and repeated activations can result in adaptation [43]. Through the application of a single acidic perfusion, we were able to minimize these effects. Moreover, the resting state networks identified through ICA point to intriguing group differences in the central response to esophageal stimulation in subjects with and without obesity. Further studies including both groups are still needed to understand the complex interactions of the brain–esophagus axis [15].

We acknowledge that the study had some limitations. The task analysis was not properly designed to generate fMRI output, since each esophageal stimulus was presented only once to participants, in a single fMRI session, as well as for a long time (300 sec). Regarding trFC analysis, we did not account for age and sex in our model; since these are important confounders in neuroimaging studies and our study sample was heterogeneous, this may have influenced the results. There was a significant age difference between the groups. However, the age difference was just 5.8 years, less than in studies that reported age-dependent changes in functional connectivity within the anterior salience network [44,45]. The periods of perfusion were short (only 5 min), and the severity of heartburn was not quantified, limiting the assessment of esophageal sensitivity. Nevertheless, the inclusion of different GERD phenotypes in young adult participants with and without obesity translates into a common type of patient in clinical practice, increasing the generalizability of our results.

In summary, in the presence of reflux symptoms, esophageal sensitivity evoked by acid perfusion was equal in subjects with and without obesity in terms of heartburn perception, but accompanied by specific changes in brain responses, especially in those with obesity. Further research into this matter is warranted to explore possible clinical applications of the findings.

Summary Box

What is already known:

- The prevalence of gastroesophageal reflux disease (GERD) is higher in the population with obesity
- Functional magnetic resonance imaging (fMRI) has been used to assess the brain–esophagus axis and the relationship with the perception of heartburn in volunteers and patients with GERD
- Evaluation of the brain–esophageal axis is lacking among patients with obesity

What the new findings are:

- In the presence of reflux symptoms, brain activation is lower in frontal areas
- Heartburn perception during fMRI does not differ between subjects with and without obesity
- Obesity is related to lower connectivity within the anterior salience network

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