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Serum levels of ghrelin and obestatin in children with symptoms suggestive of delayed gastric emptying of unclear etiology

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Abstract

Background Ghrelin and obestatin are peptides of the gut-brain axis affecting appetite and gastrointestinal motility.

Methods We conducted a cross-sectional, case-control study to determine pre- and postprandial serum levels of total ghrelin and obestatin along with gastric emptying scintigraphy in children with symptoms suggestive of delayed gastric emptying time (GET), not attributable to any identifiable cause.

Results Twenty children with symptoms suggestive of delayed GET, of whom 9 had delayed GET, and 20 age-matched healthy children were enrolled. Preprandial ghrelin and obestatin were higher compared to controls (GHR mean level in patients and controls: 1162 pg/mL and 401 pg/mL respectively; P<0.05; OB mean level in patients and controls: 417 pg/mL and 325 pg/mL respectively; not statistically significant). Postprandial ghrelin was significantly decreased in the subgroup of patients with delayed GET (GHR mean level in children with normal and prolonged GET: 1237 pg/mL and 584 pg/mL respectively; P<0.05).

Conclusion Obestatin and ghrelin were deranged in children with symptoms indicative of delayed GET of unexplained etiology. Gastric emptying was prolonged in almost half of the patients thus gastric emptying scintigraphy should be considered in the investigation of children with such symptomatology.

Keywords Children, ghrelin, obestatin, delayed gastric emptying, gastric emptying scintigraphy

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Introduction

Ghrelin (GHR) and obestatin (OB) are peptides secreted by the stomach [1]. The gene of human GHR is located on chromosome 3p26-p25 and encodes a polypeptide called preproghrelin which undergoes a stepwise proteolytic cleavage generating GHR and subsequently OB [2]. GHR is orexigenic and promotes gastrointestinal (GI) motility whereas OB exerts anorexigenic effects, decelerates gastric emptying time and small bowel activity and reduces body weight [3].

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Conflict of Interest: None

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Early satiety, bloating, nausea and vomiting are symptoms frequently encountered in pediatrics; delayed gastric emptying time (GET) has been implicated as an underlying pathophysiological mechanism for the abovementioned symptoms [4,5].

We hypothesized that GHR and OB are implicated in the pathogenesis of delayed GET, in the absence of mechanical gastric outlet obstruction, in children presenting with symptoms of delayed GET that persist >6 months and for which no cause was identified [6,7].

The aim of this study was to assess GET as well as pre- and postprandial levels of GHR and OB and examine their association.

Patients and methods

Study subjects

All children referred for further investigation at a tertiary center (Pediatric Gastronterology Unit; 4th Pediatric Department, Papageorgiou Hospital; Aristotle University of Thessaloniki; Greece) for symptoms (duration >6 months)

indicative of delayed GET between March 2007 and March 2013 were included in the study.

Twenty healthy, age-matched children without any GI symptoms or evidence of other organic disease were also recruited as controls among patients examined at the pediatric outpatient clinic for routine assessment. Inclusion and exclusion criteria are shown in (Table 1).

This study had institutional approval and was conducted according to Helsinki Declaration. Written informed consent was obtained from all study participants.

Assessment for potential organic disorders

All children of the present cohort were investigated for the presence of organic diseases with detailed clinical history, careful physical examination and a number of laboratory tests. The latter included complete blood count, baseline biochemistry with liver, pancreatic and renal function tests, C-reactive protein, erythrocyte sedimentation rate, urinalysis with culture, stool microscopy, and screening for celiac disease (total IgA and IgA anti-tissue transglutaminase antibodies) and *H. pylori* infection (serum anti-*H. pylori* IgG and IgM antibodies). Mechanical upper GI obstruction or anatomical abnormality was excluded in all patients with a combination of upper GI contrast series and/or esophagogastroduodenoscopy (EGD).

Gastric emptying scintigraphy

After an overnight fast children underwent a 2-h gastric emptying scintigraphy with a standardized meal of solids. The meal consisted of 2 AA scrambled eggs labeled with technetium 99m (^{99m}Tc) sulfur colloid (1 mCi), two thin slices of white bread, 1 teaspoon of butter (5 g) and 150mL of water to ease the ingestion process as the patients had to consume the aforementioned meal within 10 min. The amount of the test meal was calculated according to patient body surface area (test meal in g/1.73 m^2).

Table 1 Inclusion and exclusion study criteria

Inclusion criteria • Age <14 years • Symptoms present ≥6 months • No evidence of organic disease despite extensive diagnostic work-up
Exclusion criteria • Presence of gastric outlet obstruction and/or upper GI anatomical abnormality • Presence of other GI symptoms (e.g. swallowing disorders, dysphagia, gastro-esophageal reflux, abnormal bowel habits) • Previous abdominal surgery and/or other major surgery • Evidence of organic disease (present or past) which could account for the patient's symptomatology (e.g. food allergy, hypothyroidism) • Inability to ingest the gastric scintigraphy meal within 10 min or total inability to receive it (e.g. egg allergy)

GI, gastrointestinal

Immediately after meal ingestion all children were placed in the upright position in front of a γ-camera with parallel-hole collimator (Vertex-Plus Epic, ADAC/Philips). Anterior and posterior abdominal images were acquired at 15 min intervals for 1 min for a total duration of 2 h. Areas of interest were subsequently set in the stomach area and the rate at which the radioactive material left the stomach was calculated based on the geometric mean.

Gastric half-emptying time ($T_{1/2}$) was defined as the time by which 50% of the radiolabeled meal of solids had left the stomach. GET was considered delayed if $T_{1/2}$ was >90 min.

In the intervals of image acquisition children were allowed to walk outside the γ-camera room; however, and until the test was over, they were neither allowed to eat or drink nor to engage in intense play.

Blood sample collection and analysis

Venous blood samples (4 mL) were collected between 08:00-09:00 am immediately prior to the ingestion of the meal for the scintigraphic gastric emptying study and 2 h later, upon completion of the test. The blood samples were collected into biochemistry tubes (not containing EDTA, aprotinin or hydrochloric acid), and were immediately centrifuged at 4° C for 5 min at 3000 rpm. Sera were subsequently extracted and stored at -24° C.

Serum total GHR and OB were measured using the human GHR and OB RIA commercially available kits (Phoenix Pharmaceuticals, Inc., Burlingame, California, USA). Due to ethical reasons gastric emptying scintigraphy was not performed and only morning fasting blood samples were collected for the measurement of preprandial levels of total GHR and OB in the control group.

Statistical analysis

Patient characteristics, serum levels of GHR and OB, GHR/OB ratio and GET $T_{1/2}$ are expressed as mean ± standard deviation and as median and range depending on the normality of the distributions of continuous variables as assessed with Kolmogorov-Smirnov test.

Comparisons of preprandial GHR and OB as well as GHR/OB ratio between patients and controls were performed with Student's *t*-test, whereas paired *t*-test was used for comparisons between pre- and postprandial levels. Finally, *t*-test was also used to compare both pre- and postprandial levels between patients with normal and delayed GET. Categorical variables were compared using chi-square test. Furthermore the adjusted effect of different factors [gender, age, body mass index (BMI z-score)] on GET, levels of GHR and OB, GHR/OB (pre- and postprandial) was explored using multiple regression analysis. Statistical analyses were performed with SPSS version 17.0 software (SPSS Inc, Chicago, IL). P values <0.05 were considered statistically significant.

Results

Demographics and clinical characteristics

Twenty prepubertal children (7 male, 35%) with symptoms suggestive of delayed GET (early satiety, postprandial fullness, nausea, vomiting) that remained of unexplained etiology (duration of symptoms >6 months) were identified and included in the study. Of note, the initial diagnostic work-up which included a complete blood count, baseline biochemistry with liver, pancreatic and renal function tests, C-reactive protein, erythrocyte sedimentation rate, urinalysis with culture, stool microscopy, celiac disease (total IgA and IgA antitissue transglutaminase antibodies) and *H. pylori* (serum IgM and IgG antibodies) screening was within normal range. Furthermore, no mechanical upper GI obstruction or anatomical abnormality was revealed in any patient by means of a combination of upper GI contrast series and/or EGD. Of note, the histopathology of the biopsies taken during all EGD procedures was also without any abnormalities. Demographic data and baseline patient characteristics are presented in (Table 2). Twenty healthy, age-matched controls were also included.

GET

Nine of 20 patients had delayed GET ($T_{1/2}$ >90 min). The median time for gastric emptying rate was 80.5 (range: 55-210) min. GET was not significantly affected by age, gender and BMI (adjusted effect as assessed with regression analysis).

Table 2 Study participants' clinical characteristics and laboratory findings

Variable	Patients	Controls
Number	20	20
Median age, years (range)	6 (2-12)	8 (2-12)
Gender ratio, male: female	7:13	10:10
BMI z-score (±SD)	−1.99±1.79	0.24±0.69
Symptoms (number of patients, %) Early Satiety Bloating Failure to thrive Occasional nausea/vomiting	 20 (100) 11 (55) 12 (60) 4 (20)	 N/A
GHR preprandial (pg/mL; mean±SD)	1161.9±760.3	401.1±254.6
GHR postprandial (pg/mL; mean±SD)	943.3±564.1	N/A
OB preprandial (pg/mL; mean±SD)	416.7±110.2	325.3±163.6
OB postprandial (pg/mL; mean±SD)	535.1±155.2	N/A
GHR/OB preprandial (mean±SD)	2.8±1.5	1.3±0.6
GHR/OB postprandial (mean±SD)	1.7±0.8	N/A
GET T1/2 (min; median, range)	80.5 (55-210)	N/A

N/A, not applicable; SD, standard deviation; BMI, body mass index; GHR, ghrelin; OB, obestatin; GET, gastric emptying time; GET $T_{1/2}$, gastric half-emptying time

Serum total GHR and OB levels

The preprandial levels of GHR, OB and the GHR/OB ratio were significantly higher in patients compared to controls (P<0.05) (Fig. 1). Postprandially (patient group) there was a decrease in GHR and an increase in OB levels which were not statistically significant; however the postprandial GHR/OB ratio was found to be significantly lower (Fig. 2). Moreover, the postprandial levels of GHR and the GHR/OB ratio (but not OB) were significantly lower in the patients who had delayed GET (P<0.05) (Fig. 3). Pre- and postprandial levels of GHR, OB were not significantly affected by patient/control characteristics (adjusted effect, regression analysis).

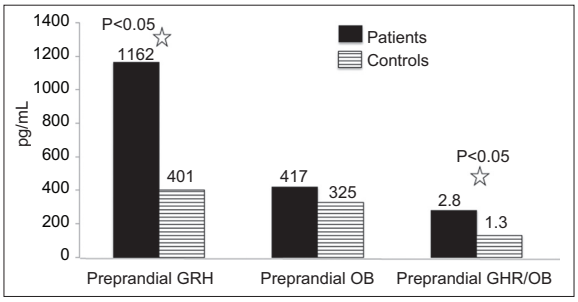

Figure 1 Preprandial levels of GHR, OB and GHR/OB ratio in patient and control groups
GHR, ghrelin; OB, obestatin; GHR, OB and GHR/OB values represent mean values

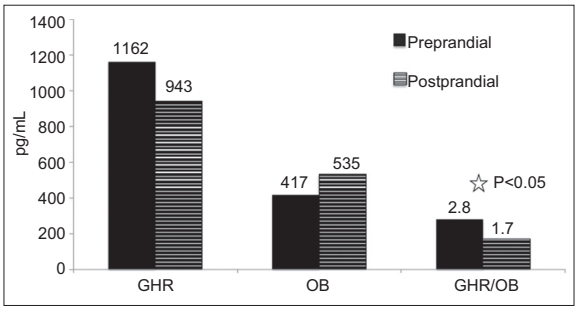

Figure 2 Pre- and postprandial levels of GHR, OB and GHR/OB ratio in patient group
GHR, ghrelin; OB, obestatin; GHR, OB and GHR/OB values represent mean values

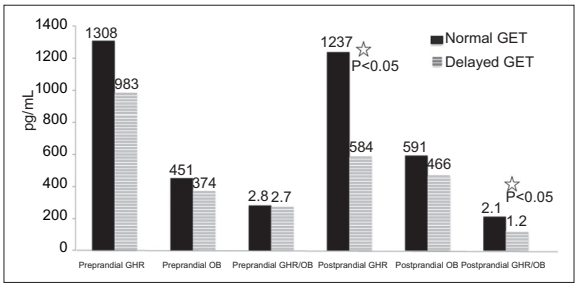

Figure 3 Pre- and postprandial levels of GHR, OB and GHR/OB ratio in patients with normal and delayed gastric emptying time (GET)
GHR, ghrelin; OB, obestatin; GHR, OB and GHR/OB values represent mean values

Discussion

In the present study we assessed GET and total GHR, OB serum levels in children with persistent (>6 months) and unexplained symptoms of delayed gastric emptying. We found that: GET was prolonged in 9 out 20 patients; preprandial GHR and OB levels were significantly higher in patients than controls; patients with delayed GET had significantly lower postprandial GHR and GHR/OB ratio compared to those with normal GET.

Approximately 50% (9/20) of the patients had delayed GET of solids as assessed with the gold standard method of scintigraphic imaging of a radiolabeled meal [8,9]. This finding is in line with results published by other researchers and highlights the fact that delayed GE is possibly implicated in the pathogenesis of dyspeptic symptoms [10]. Interestingly, in the other 50% of the patients GET was normal indicating that another cause should be sought for their symptomatology.

The preprandial levels of GHR and OB were higher in our patients as compared to controls. This is an interesting finding which is in contrast with the lower preprandial GHR, OB levels demonstrated by other researchers in adults with functional dyspepsia [11]. Our results however are similar with studies in children with poor appetite and adults with anorexia nervosa (AN) which revealed increased preprandial levels of GHR and OB particularly in AN patients compared to healthy subjects [12-14].

Of note, Ang *et al* have demonstrated that GHR increases the gastric tone in the fasting state and inhibits gastric accommodation when administered during a meal [15]. The latter could possibly explain the symptoms of early satiety and postprandial fullness in the patients of the present study who had significantly higher preprandial GHR levels.

Finally, we have shown that the postprandial levels of GHR and the GHR/OB ratio are significantly lower amongst patients with delayed GET. Limited data exist on the correlation between GHR and GET in patients with dyspeptic symptoms. Lee *et al* did not find a significant correlation between pre-/postprandial GHR and GET in adults with dysmotility-like functional dyspepsia, although 5 out 7 patients with abnormally low preprandial GHR had delayed GET [11]. Noteworthily, postprandial OB was found to increase in our patients however this was not statistically significant. The resulting imbalance in the GHR/OB ratio may be implicated in the pathogenesis of dyspeptic symptoms, although further research is required to validate this hypothesis [12,13].

The interplay between gut peptides such as GHR, OB and GI motility is still the subject of research, however due to the scarce data our results are not easily interpreted [11,15,16].

We have measured total GHR, which is considered as an acceptable surrogate for acylated GHR, and not its isoforms (acyl- and des-acyl-GHR) which have opposite biologic properties (acyl-GHR: orexigenic, promotes GI motility, des-acyl-GHR: opposite effects) [3,11,17-19]. A potential imbalance between the two could also partially explain our results.

Polymorphisms in the preproghrelin gene, differences in the transcriptional, translational and post-translational processes could also lead to disturbances in the production of GHR and OB [13,14,20,21]. Moreover the role of sequence variants in the coding region of GHR receptor (growth hormone secretagogue receptor - GHSR1a) and genetic variations of the enzyme responsible for its acylation and thus biologic activity (GHR O-acyltransferase, GOAT) have also been explored in the regulation of body weight in children and in adults with AN, without, however, definitive conclusions [22,23].

Limitations of the present study include the small number of patients and the fact that total serum GHR was measured without discrimination between acyl-GHR which promotes the appetite and GI motility and des-acyl-GHR that has biological properties similar to OB [1]. Moreover, postprandial serum levels of GHR, OB along with scintigraphic evaluation of GET have not been assessed in controls as this was not ethically acceptable. Furthermore amongst the various parameters of the gastric sensorimotor function that could cause symptoms similar to the ones described, GET was the only one assessed, as other diagnostic modalities, e.g. electrogastrogram and barostat, were not available in our institution.

Strengths of the study include the homogeneous and carefully selected population and the fact that it offers a novel insight regarding the role of GHR and OB in children with GI symptoms.

In conclusion, our data suggest that delayed gastric emptying is common in children with unexplained dyspeptic symptoms. In these patients, GHR and OB are deranged suggesting that they may play a role, not as yet fully clarified, in the regulation of gastric motility and the pathogenesis of such symptoms.

Summary Box

What is already known:

- Ghrelin and obestatin are peptides of the gut-brain axis affecting appetite and gastrointestinal motility
- Delayed gastric emptying has been implicated in the pathogenesis of dyspeptic symptoms (e.g. early satiety, bloating)
- The complex mechanisms regulating gastric motility in children are still not well understood

What the new findings are:

- Delayed gastric emptying was not uncommon in children with unexplained dyspeptic symptoms
- Serum levels of ghrelin and obestatin were deranged in children with symptoms suggestive of delayed gastric emptying not attributable to any identifiable cause
- Ghrelin to obestatin ratio seems to affect gastric emptying time in children with the aforementioned symptomatology

Further studies are warranted to fully understand their role, particularly in view of the therapeutic potential of ghrelin in the treatment of gut motility disorders.

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