

Ghrelin in patients with irritable bowel syndrome

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Abstract. General gastrointestinal dysmotility occurs in patients with irritable bowel syndrome (IBS). Ghrelin seems to play an important role in regulating gastrointestinal motility. The present study was undertaken, therefore, to establish the possible role of ghrelin in the pathophysiology of IBS. Thirty-seven patients with IBS (19 had IBS-constipation and 18 IBS-diarrhoea) were included in this study. Ten healthy volunteers served as controls. After overnight fast, blood samples were drawn from patients and controls, and a gastro-duodenal endoscopy was performed. Biopsies were taken from oxyntic mucosa and duodenum. Ghrelin cell density was determined by computer image analysis after immunohistochemical staining of the tissues. Total and active ghrelin were detected in tissue extracts and plasma by commercially available RIA and ELISA Kits. The density of ghrelin-immunoreactive cells in the oxyntic mucosa was significantly lower in IBS-constipation and significantly higher in IBS-diarrhoea patients than healthy controls ($P < 0.0001$ and < 0.0001 , respectively). There was no statistical difference in total or active ghrelin between IBS patients and controls, regarding tissue extracts or plasma. In order to compensate for the increase and decrease in the ghrelin cell density, the synthesis and release of ghrelin may be decreased and increased in IBS-diarrhoea and IBS-constipation patients, respectively. It has been speculated that this compensatory mechanism may be subjected from time to time to fatigue with the subsequent increased and decreased synthesis and release of ghrelin in IBS-diarrhoea and IBS-constipation with a subsequent intermittent diarrhoea or constipation seen in these patients, respectively.

Introduction

Approximately 15 to 25% of the world population suffers from irritable bowel syndrome (IBS) (1). IBS is a chronic

condition, which is characterized by abdominal discomfort or pain, abdominal bloating and changes in bowel habit (1,2). The degree of symptoms varies in different patients from tolerable to severe, interfering with daily activity. IBS is the most common diagnosis in gastroenterology and is estimated to be 20-40% of all consultation performed by gastroenterologists (2,3). Besides the increased morbidity caused by IBS, it represents an economic burden to the society in different indirect forms such as increased sick leave and over consumption of healthcare resources (2,3). General disturbances in gastrointestinal motility have been reported in patients with IBS (4-10). The cause of gastrointestinal dysmotility has been speculated to be a result of genetic, psychosocial factors and stress (1-3).

Ghrelin is a 28-amino acid peptide hormone, which was isolated from the stomach (11). Ghrelin originates mostly from endocrine cells in the oxyntic mucosa of the stomach but small amounts were found in both the small intestine and arcuated nucleus of the hypothalamus (11,12). Ghrelin has several functions, the most known is its growth hormone (GH)-releasing effect in the pituitary, where it acts synergistically with GH-releasing hormone (11,13). Ghrelin also increases appetite and feeding and plays a major role in energy metabolism (14,15). Furthermore, ghrelin has been found to accelerate gastric as well as small and large intestinal motility (16-26).

As ghrelin seems to play an important role in regulating gastrointestinal motility and IBS patients exhibit gastrointestinal dysmotility, the present study was undertaken to establish the possible role of ghrelin in the pathophysiology of IBS.

Materials and methods

Patients and healthy subjects. Thirty-seven patients with irritable bowel syndrome (IBS) according to the Rome III criteria (35 females and 2 males; mean age 41 years; range 22-60) were included in this study. These patients did not show any alarming symptoms. The patients were subdivided into 2 subtypes according to Rome III criteria. Thus, 19 patients had IBS-constipation (all females; mean age 38 years; range 24-57) and 18 patients with IBS-diarrhoea (16 females and 2 males; mean age 44 years; range 22-60). Ten healthy volunteers without any gastrointestinal complaints (8 females and 2 males; mean age 32 years; range 21-49) served as controls. The study was performed in accordance with the Declaration of Helsinki and was approved by the Local

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Committee for Medical Research Ethics. All subjects gave oral and written consent. After overnight fast, blood samples were drawn from patients and controls, and a gastroduodenal endoscopy was performed.

Gastrointestinal endoscopy. During the endoscopy procedure, 9 biopsies were taken from the corpus (major curvature) and pars descendens duodeni (distal to papilla of Vateri). In addition, biopsies were taken from the antrum and used in the CLO test for *Helicobacter pylori*.

Histopathology and immunohistochemistry. Three of the biopsies taken from the corpus and from the duodenum were fixed in 4% buffered paraformaldehyde overnight, embedded in paraffin and cut into 5- μ m sections. The sections were stained by haematoxylin and eosin and immunostained with the avidin-biotin-complex (ABC) method (Dako Cytomation, Glostrup, Denmark) as previously described in detail (27). The primary antiserum used was anti-ghrelin (polyclonal, code no. 00182, dilution 1:1600, Phoenix Pharmaceuticals, Belmont, CA, USA). The sections were counterstained slightly with haematoxylin. Negative controls included replacing the primary antiserum with 1% bovine albumin and by pre-incubating the diluted antiserum with excessive ghrelin (50 μ g/ml, NeoMPS, Strasbourg, France) at 4°C overnight.

Computerized image analysis. This was performed using Leica's Quantimet 600MC Image Processing and Analysis System (Leica, Cambridge, UK) linked to an Olympus microscope, type BX50. The program used in this system was QWIN, Leica's Windows Based Image Analysis Tool Kit, version 2.6. The system included QUIPS (version 2.6), which is an interactive programming system. When using x20 objectives, each pixel in the computer monitor corresponds to 0.173 μ m and the frame (fields) representing an area of 5436 μ m². The number of ghrelin-immunoreactive cells and the area of the epithelial cells were measured. Using QUIPS, an automated standard sequence analysis operation was created, as described previously in detail (28). Briefly, the number of immunoreactive cells was counted using the field measurements. The areas of the epithelial cells were measured using a threshold setting. Data from each field were tabulated, the number of cells/mm³ were computed and statistically analysed automatically. Measurements were carried out in 10 randomly chosen fields in the stomach and duodenum of each individual. The x20 objective was used.

Detection of ghrelin. The blood samples were collected in pre-chilled polypropylene tubes containing EDTA (1 mg/ml blood) and aprotinin (500 U/ml blood) and were then immediately centrifuged at 4°C for 10 min at 3000 rpm. The plasma fraction was collected and diluted 1/10 volume with 1 N HCl and stored at -20°C until analysis. The mean weight of the biopsies obtained from the gastric corpus was 67.7 mg (range 102.6-36.6) and from the duodenum, 64 mg (range 96.8-30.6). The tissue from the stomach and duodenum were quickly frozen and stored at -80°C until the time of extraction. Each tissue sample was diced and boiled for 10 min in 2 ml distilled water to inactivate intrinsic proteases. The solution was adjusted to 1 M acetic acid after cooling, and the tissue

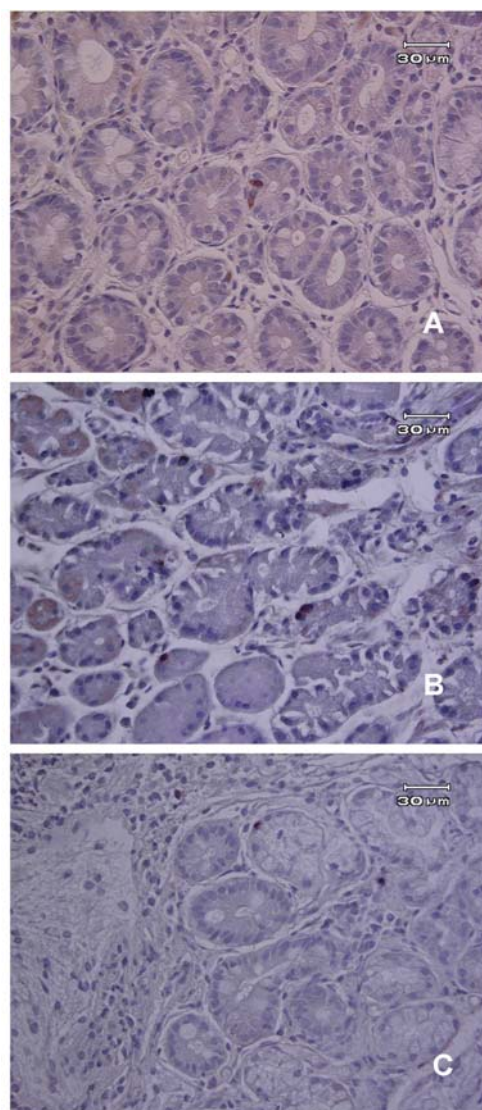


Figure 1. Ghrelin-immunoreactive cells in the oxyntic mucosa of a control (A), a patient with IBS-diarrhoea (B) and a patient with IBS-constipation (C).

was homogenized with a Polytron mixer. The supernatant was obtained after centrifugation at 10,000 rpm for 30 min, and then stored at -80°C until the time of analysis.

Total ghrelin (acylated and des-acylated) was measured with a commercially available Radioimmunoassay (RIA) Kit (Phoenix Pharmaceuticals, Inc.). The active (acylated) ghrelin was detected by a commercially available ELISA Kit (Sceti Co., Ltd., Tokyo, Japan).

Statistical analysis. The Kruskal-Wallis non-parametric ANOVA test and Dunn's post-test were used to compare controls and the 2 subgroups of IBS patients. P-values <0.05 were considered significant.

Results

Gastrointestinal endoscopy. The stomach and duodenum of both patients and the healthy volunteers were macroscopically normal. Three patients and one healthy volunteer exhibited a positive CLO test, indicating infection with *H. pylori*.

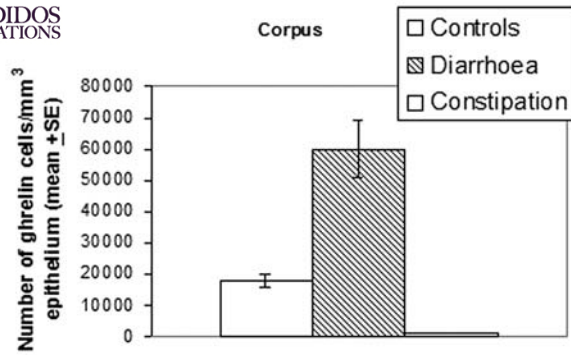


Figure 2. Density of ghrelin-immunoreactive cells in the oxyntic mucosa of the stomach in healthy subjects and in patients with IBS. *** $P > 0.0001$.

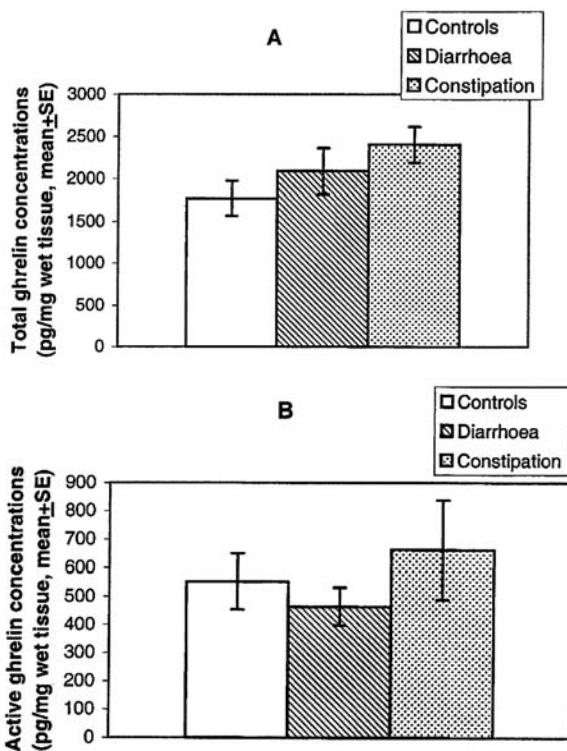


Figure 3. Concentrations of total (A) and active (B) ghrelin in tissue extracts from the oxyntic mucosa of controls and patients.

Histopathology and immunohistochemistry. Histopathological examination of the gastric and duodenal biopsies from patients and the healthy volunteers revealed normal histology. Ghrelin-immunoreactive cells were found in the stomach oxyntic mucosa and among the epithelial cells of the duodenum. In the duodenum, ghrelin cells were few and unevenly distributed which did not allow any reliable quantification. They occurred mostly in the crypts. Ghrelin cells were round, flask-shaped or triangular (Fig. 1).

Computerized image analysis. The density of ghrelin-immunoreactive cells in the oxyntic mucosa of patients with IBS-diarrhoea was significantly higher ($P < 0.0001$) and this density in patients with IBS-constipation was significantly lower than healthy controls ($P < 0.0001$) (Fig. 2).

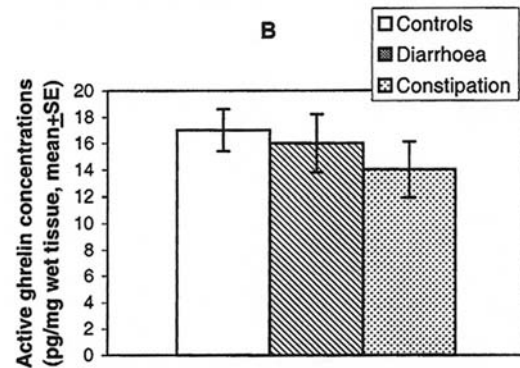
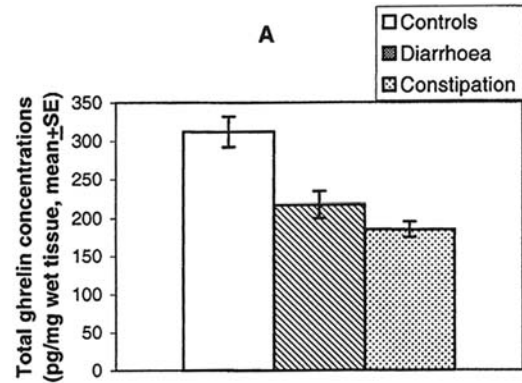


Figure 4. Concentrations of total (A) and active (B) ghrelin in tissue extracts from the duodenal mucosa of controls and patients.

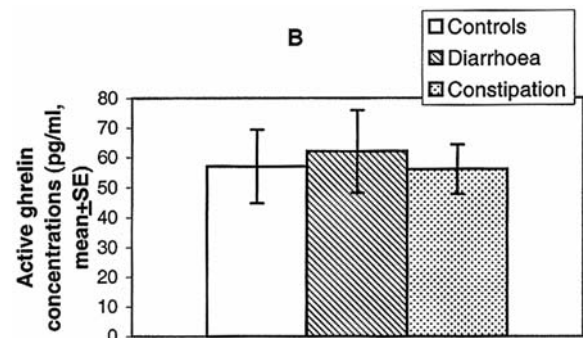
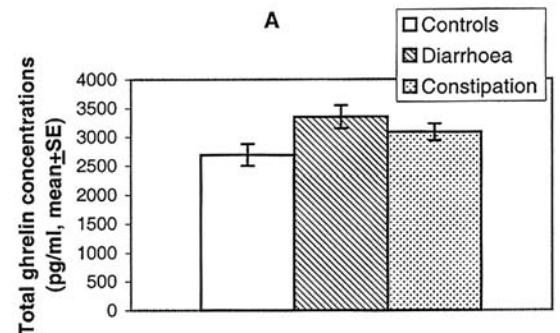


Figure 5. Plasma concentrations of total (A) and active (B) ghrelin in controls and patients.

Detection of ghrelin. There was no statistical difference between controls and patients regarding the concentrations of total and active ghrelin in the corpus, duodenum and plasma (Figs. 3-5).

Discussion

In the present study, ghrelin was found to be affected in patients with IBS. Ghrelin has been reported to be abnormal in other gastrointestinal diseases such as inflammatory bowel diseases (29), coeliac disease (30-33) and atrophic gastritis (34-36). Thus, ghrelin seems to be involved in the pathophysiology of gastrointestinal diseases/disorders.

The present study showed that ghrelin cell density in the oxyntic mucosa of patients with IBS was abnormal. The nature of this abnormality was, however, different in the different subtypes of IBS. Thus, whereas ghrelin cell density increased in IBS-diarrhoea patients, it was reduced in IBS-constipation patients. The concentrations of total and active ghrelin in extracts of the oxyntic mucosa and of the duodenum of IBS patients did not differ from those of healthy controls. There was no difference either in the plasma levels of total and active ghrelin between IBS patients and controls. It is conceivable, therefore, to conclude that in order to compensate for the increase and decrease in the ghrelin cell density, the synthesis and release of ghrelin decreased and increased in IBS-diarrhoea and IBS-constipation patients, respectively.

One may speculate further, that this compensatory mechanism in IBS patients may be subjected to fatigue from time to time with the subsequent increased and decreased synthesis and release of ghrelin in IBS-diarrhoea and IBS-constipation, respectively. As ghrelin accelerates gastrointestinal motility (16-26), that would account for the intermittent diarrhoea and constipation noted in these patients, respectively.

The present findings in the oxyntic mucosa of increased ghrelin cell density in IBS-diarrhoea and the paucity of ghrelin cells in IBS-constipation patients raise the question: whether ghrelin antagonist and ghrelin would have a therapeutic role in these patients.

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