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Ghrelin, pancreatic polypeptide plasma concentrations and gastric myoelectric activity in celiac disease

Magdalena Przybylska-Feluś[1], Agata Furgała[2], Jolanta Kaszuba-Zwoińska[2], Piotr Thor[2], Tomasz Mach[1], Małgorzata Zwolińska-Wcisło[1,3]

[1]Department of Gastroenterology and Hepatology, Jagiellonian University Medical College
ul. Śniadeckich 5, 31-531 Kraków, Poland
[2]Department of Pathophysiology, Jagiellonian University Medical College
ul. Czysta 18, 31-121 Kraków, Poland
[3]Department of Clinical Dietetics, Jagiellonian University Medical College
ul. Śniadeckich 5, 31-531 Kraków, Poland

Corresponding author: Małgorzata Zwolińska-Wcisło, MD, PhD
Chair of Gastroenterology, Hepatology and Infectious Diseases, Jagiellonian University Medical College
ul. Śniadeckich 5, Kraków, Poland; Phone/Fax: +48 12 424 73 80, +48 12 424 73 40
E-mail: mzwcislo@su.krakow.pl

Abstract: Background / Aims: The aim of the study was to analyze the effect of celiac disease (CED) on the upper-gut motility and release of enteral hormones (ghrelin and pancreatic peptide (PP)).
Materials and methods: The study included 25 patients diagnosed with CED and 30 healthy controls. Gastric myoelectric activities (EGG) in a fasted and fed state were recorded. The plasma concentrations of ghrelin and PP were determined.
Results: CED patients presented in a fasted state a decreased percentage of normogastria 54.8 ± 24.5 vs. 86 ± 12.3%, p = 0.02 and slow wave coupling (SWC) 52.7 ± 13.4 vs. 77.4 ± 11.9%; p = 0.00001 with increased dominant power (DP) 11.6 ± 1.5 vs. 11.1 ± 1.1. Contrary to the controls, they did not show an improvement in the percentage of normogastria, DP and SWC when examined in a fed state (p <0.05). Furthermore, CED patients presented with significantly lower fasting plasma concentrations of ghrelin 156.8 ± 86.7 vs. 260.2 ± 87.6 pg/ml, p = 0.0002 and significantly higher fasting PP levels than did the controls 265.2 ± 306.3 vs. 54.1 ± 54.6 pg/ml, p = 0.0005.
Conclusion: CED affects gastric myoelectric activity (decreasing normogastria and coupling) and causes changes in fasting concentrations of enteral hormones (decrease in ghrelin and an increase in PP). Gastric myoelectric response to food is abolished in CED patients, probably due to the neurohormonal changes induced by primary inflammation associated with this disease.

Key words: celiac disease, ghrelin, pancreatic polypeptide, gastric myoelectric activity.

Introduction

Celiac disease (CED) is an immune-mediated enteropathy triggered by gluten exposure among susceptible subjects [1–4]. It affects approximately 0.3–2.4% of European population [1]. Clinical outcome comprises both intestinal and extra-intestinal manifestations. The most typical, albeit nonspecific, symptoms of CED include diarrhea, abdominal pain, dyspepsia, flatulence, malabsorption and weight loss. Extra-intestinal manifestations may comprise neurological dysfunction in form of polyneuropathy or autonomic impairment [3].

About 30–60% of CED patients present with symptoms that likely correspond to gastrointestinal (GI) motility disorders [4]. Alterations of gastrointestinal motility observed in patients with CED include impaired esophageal transit, delayed orocecal transit time and faster colonic transfer [5, 6]. These abnormalities result from malabsorption, autonomic disturbances, enteral hormone derangement and interactions thereof [5]. Untreated CED patients were shown to present with a delayed gastric emptying, which eventually normalized after gluten withdrawal [5, 6].

Gastric motility may be examined by means of gastric emptying measurement or gastric myoelectric activity recording (electrogastrography, EGG). While the former technique can be used solely to determine the postprandial motor status, the latter accurately determines gastric motor function in both fed and fasted state [7, 8]. Parallel evaluation of gastric emptying and myoelectric activity, with an analysis of a relationship between clinical features and characteristics of the recorded gastric dysmotility, was a subject of few previous studies in patients with various disorders [6, 8–10]. Delayed gastric emptying was shown to be linked to EGG changes, namely a lower percentage of normal gastric slow waves and a lower postprandial increase in dominant power (DP) of EGG. A canine study demonstrated that while each gastric slow wave is accompanied by a contraction, the latter is lost in the case of a dysrhythmia; furthermore, a relative increase in EGG DP was shown to be associated with an increase in gastric contractile activity [11]. An increase in the percentage of normal slow waves, dominant frequency (DF) and dominant power, as well as a concomitant decrease in the parameters of dysrhythmia, are typically observed in healthy subjects as a gastric myoelectric response to food [11].

Ghrelin and pancreatic polypeptide (PP) among other functions play crucial roles in enterohormonal regulation of gastrointestinal motility. Ghrelin is a peptide with multiple functions, among others being involved in the control of GI motility, acid secretion and regulation of food intake; furthermore, it exerts an anti-inflammatory effect in the GI mucosa [12]. Plasma concentration of ghrelin is decreased in the case of positive energy balance and increases during starvation [13]. Ghrelin stimulates both food intake and GI motility and suppresses insulin secretion. It induces

premature phase III contractions and its release is believed to be mediated by the vagal stimulation [14]. Several GI disorders were shown to be associated with an impaired secretion of ghrelin [12]. Due to the role of ghrelin in the control of food intake, any disruptions of this hormone result in an impairment of GI motility and gastric acid secretion [12, 15]. However, the available data on plasma concentration of ghrelin in CED are inconclusive, probably due to the fact that the latter condition is associated with malnutrition and motility disturbances [16–18].

PP is secreted by the Langerhans islets (PP cells) and intestinal cells [19]. Postprandial changes in PP concentration correlate with caloric value of a meal. Following ingestion of nutrients, circulating PP concentrations increase in a biphasic manner, proportionally to caloric load, and remain elevated up to 6 h after the meal [19]. Physiological effects of PP include inhibition of gastric emptying, gallbladder motility and pancreatic exocrine secretion [19]. Moreover, an increase in the concentration of this enteral hormone was demonstrated to be linked to reduction of gastric emptying. To this date, secretion of the gut-brain axis peptide hormones in children and adults with CED was examined prior to and during the treatment [20]. Chronic inflammation of the small bowel observed in patients with CED may lead to autonomic disturbances and resultant impairment of anti-inflammatory response associated with overactivity of the vagal nerve.

Impaired motility in CED may be directly linked to changes in ghrelin and PP release, their circulating concentrations and/or receptor binding, or occur secondarily to inflammatory damage of neurochemical gastric motility coding. The available data on gastric motility changes and ghrelin response in CED patients are inconclusive and sparse. Presence of gastric pathologies may be reflected by abnormalities of EGG recordings. The food-induced changes in gastric myoelectric activity of CED patients have not been studied thus far. Furthermore, to the best of our knowledge, none of the previous studies analyzed an association between EGG recordings, ghrelin and PP concentrations in individuals with CED.

Therefore, the aims of this study were to examine the changes of gastric myoelectric activity and circulating concentrations of ghrelin and PP in patients with CED and attempt to answer what is the mechanism of the origin of these disturbances.

Materials and methods

Clinical procedure

The study included 55 participants who were divided into the following groups:

- **CED group** — 25 symptomatic patients with CED (8 men, 17 women, mean age 42.4 ± 15.8 years)
- **CG — Control group —** 30 healthy controls (9 men, 21 women, mean age 42.1 ± 9.2 years) without a history of GI disorders.

The presence of CED was confirmed on the basis of serological tests: anti-endomysial antibodies (EmA) and anti-tissue transglutaminase (tTG) antibodies and duodenal histopathology. Detailed characteristics of the study participants are presented in Table 1.

The exclusion criteria of the study were: GI disorders other than CED, diabetes mellitus, obesity (Body Mass Index — BMI ≥30kg/m^2), tobacco smoking, alcohol abuse, intake of medications with known interference with gastric myoelectric activity and autonomic function, history of abdominal surgeries or chronic disorders.

Table 1. Clinical characteristics of the study participants.

Parameters	Celiac disease (N = 25)	Control group (N = 30)	p
Age (years)	42.4 ± 15.8	42.1 ± 9.2	NS
Sex (female/male)	17/8 (68%/32%)	21/9 (70%/30%)	NS
Celiac disease duration (years)	8.8 ± 10.5 (0–40)	0	—
Marsh scale Type I Type III	 9 patients 15 patients	 0 0	—
Anti-tissue transglutaminase antibodies (U/ml)	138.5 ± 80,4	12.4 ± 6.2	—
BMI (kg/m^2)	21.7 ± 2.65	23.8 ± 0.6	NS
Albumin (g/dl)	43.6 ± 5.1	58.7 ± 4.2	<0.05
CRP (mg/l)	2.1 ± 0.4	1.2 ± 0.5	NS
Lymphocyte (10^3/µl)	1742.9 ± 849.5	2032.9 ± 946.2	NS

p <0.05 — statistically significant; NS non-significant (Student t-test)

All subjects were asked to fast for at least 12 hours prior to the study and to refrain from medications with a known effect on the autonomic function and GI motility during three days preceding the study.

Assessment of gastric myoelectric activity

Electrogastrography (EGG)

30-minute recordings of gastric myoelectric activity (EGG) under basal conditions were obtained in both groups after an overnight fast and one hour after a standard meal (Nutridrink, Nutricia, 300 kcal/300 ml). The EGG was conducted with a four-channel electrogastrography Polygraf NET (Medtronic, USA) as described by Thor *et al.* [21].

The following EGG parameters were evaluated: percentage of dysrhythmia time (0.5–2 cycles per minute (cpm) — bradygastria; 4–10 cpm — tachygastria),

percentage of normogastria (2–4 cpm), dominant frequency (DF), dominant power of dominant frequency (DP) and percentage of time with slow-wave coupling between 2–3 channels (SWC). Period Dominant Frequency (DF) was calculated as an average value for the dominant frequencies (highest peak) of FFT (Fast Fourier Transform) line. Period Dominant Power (DP) was defined as the power or amplitude of the DF peak. Changes in the EGG dominant power likely reflect gastric contractility [21, 22]. Gastric myoelectric activity includes slow waves (referred to as electrical control activity) and spike potentials associated with electrical response activity [11]. The frequency of normal gastric slow waves (normogastria) in humans corresponds to approximately 3 (2 to 4) cpm and lasts >70% of time. Potential deviations from normogastria include gastric dysrhythmias (bradygastria, tachygastria and arrhythmia), electro-mechanical uncoupling and abnormal slow wave propagation [11, 22].

Biochemical assays

The list of analyzed biochemical parameters included serum concentrations of thyroid-stimulating hormone (TSH), calcium and albumin, concentration of hemoglobin, mean corpuscular volume, erythrocyte and lymphocyte counts, INR (International Normalized Ratio) and the level of C-reactive protein (CRP) as a marker of inflammation. Serum samples were obtained shortly before the EGG recording.

Plasma concentrations of ghrelin and PP were determined at rest in a fasted state. Blood samples were stored at ice until centrifuged (3800 g at 8°C for 10 minutes) to separate the plasma as soon as possible after obtaining. Supernatant was aspirated and stored until analysis (6 h to 1 month at –20°C). Plasma concentration of ghrelin was determined with Human (Active) Ghrelin ELISA kit (Demeditec Diagnostic GmbH, Germany) for automated systems, and concentration of PP with Human Pancreatic Polypeptide ELISA kit (EMD Milipore Corporation, USA), both used in line with the manufacturers' instructions. The sensitivity of the ghrelin and PP assays was 25 pg/ml and 12.3 pg/ml, respectively.

Histopathology

Histopathological specimens were typed according to a 5-step Marsh scale which scores the presence of mucosal villous atrophy, crypt hyperplasia and intra epithelial lymphocyte effusion. Type 0 of the scale corresponds to normal mucosa, type I (infiltrative) to increased number (>25) of intraepithelial CD3+ lymphocytes per 100 enterocytes, type II (hyperplastic) to increased crypt hyperplasia with normal villi, types III A–C to CD3+ lymphocyte effusion, crypt hyperplasia and villous atrophy of various severity (from mild to total), and type IV to total villous atrophy and lack of crypt hyperplasia.

Types III A–C represent histological changes being typically associated with CED [2, 3]. Histopatological examinations were performed in Department of Pathology.

Bioethics

The study was conducted in accordance with the Helsinki Declaration and its protocol was approved by the Local Bioethics Committee (permission no. KBET/148/B/2012). All participants gave their written informed consent to participate in the study.

Statistical analysis

Statistical analysis of the results was conducted with software STATISTICA 10.0 package license for our University (StatSoft Inc., Tulsa, OK, USA). Normal distribution of each variable was verified with the Shapiro–Wilk test. Student t-test for dependent variables and Wilcoxon singed rank test were used for intragroup comparisons of normally and non-normally distributed variables, respectively. Intergroup comparisons were conducted with Student t-test for independent variables or Mann-Whitney U-test. Direction and power of relationships between pairs of quantitative variables were assessed on the basis of Spearman's coefficients of rank correlation. The threshold of statistical significance for all the tests was set at $p < 0.05$.

Results

Biochemical parameters

All participants were euthyroid and presented with normal total blood counts, CRP, calcium and albumin levels. Nevertheless, we found a significant intergroup difference in albumin concentration.

Histopathological findings

CED patients presented with histopathological changes corresponding to type I (infiltrative) and type III (A–C) characterized by intraepithelial lymphocyte effusions, crypt hyperplasia and villous atrophy (from mild to total).

EGG parameters

Compared to the controls, CED patients presented with a decreased percentage of time with normogastria and SWC with increased dominant power (log DP) when examined in a fasted state. In turn, the percentages of time with tachygastria, bradygastria and arrhythmia in CED patients were significantly higher than in the controls (Fig. 1, Table 2).

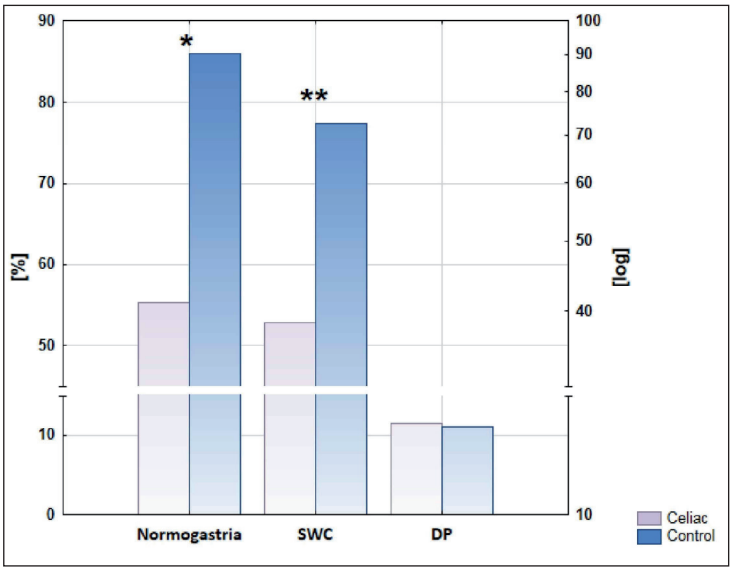

Fig. 1. Comparison of preprandial EGG parameters in patients with celiac disease and healthy controls. SWC — coupling of slow wave, DP — dominant power; * — p = 0.02; ** — p = 0.00001; statistically significant intergroup differences.

Table 2. EGG parameters determined in a fasted and fed state.

EGG parameters	Celiac disease	Control group	p
Rest (Fasting state)			
DF (cpm/min)	2.5 ± 0.6	3.0 ± 0.6	**0.009**
DP (log)	11.6 ± 1.5	11.1 ± 1.1	**NS**
Normogastria (%)	54.8 ± 24.5	86.0 ± 12.3	**0.02**
Bradygastria(%)	7.9 ± 7.3	2.6 ± 4.3	**0.001**
Tachygastria(%)	7.9 ± 7.3	2.6 ± 4.3	**0.02**
Arrhythmic(%)	29.0 ± 15.9	8.5 ± 8.5	**0.00001**
SWC (%)	52.7 ± 13.4	77.4 ± 11.9	**0.00001**
After meal (Feeding state)			
DF (cpm/min)	2.6 ± 0.7	3.1 ± 0.3	**0.002**
DP (log)	11.42 ± 1.4	11.49 ± 1.2	**NS**
Normogastria (%)	54.9 ± 20.4	87.1 ± 9.0	**0.00001**
Bradygastria (%)	7.4 ± 6.2	2.8 ± 4.1	**0.001**
Tachygastria (%)	10.1 ± 8.2	4.9 ± 4.5	**0.004**
Arrhythmic (%)	26.7 ± 16.0	5.2 ± 5.9	**0.00001**
SWC (%)	59.1 ± 14.5	82.7 ± 10.8	**0.00001**

DF — dominant frequency; DP — dominant power of dominant frequency; SWC — percentage of slow-wave coupling between 2–3 channels; NS — non-significant.

When examined in a fed state, CED patients did not show an improvement in the percentage of time with normogastria, log DP, dominant frequency and slow wave coupling. In contrast, a significant increase in log DP, DF, SWC and percentage of time with normogastria was observed in the controls when compared to the respective parameters determined in a fasted state ($p < 0.05$) (Fig. 2, Table 2). However, the disturbances of slow wave frequency did not improve after a standard meal.

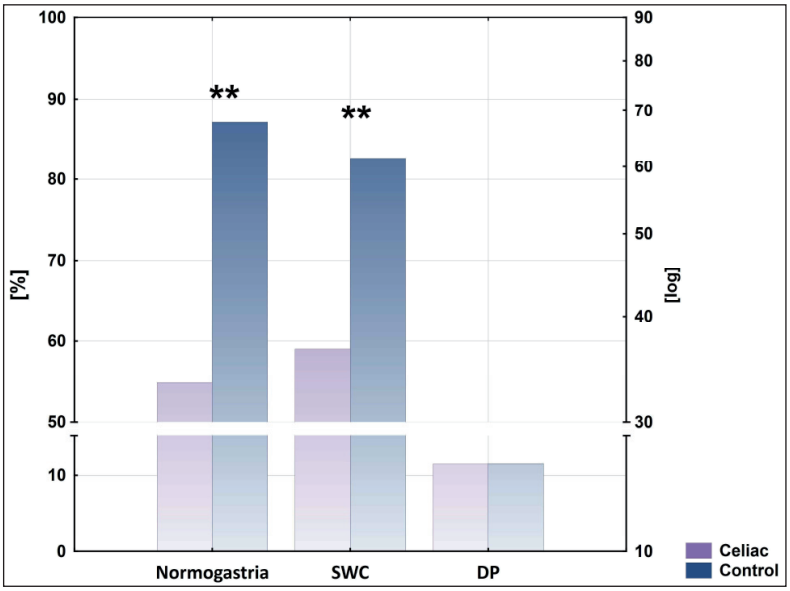

Fig. 2. Comparison of postprandial EGG parameters in patients with celiac disease and healthy controls. SWC — coupling of slow wave, DP — dominant power; ** — p = 0.00001 — statistically significant intergroup difference.

Enterohormone levels

While fasting plasma concentration of ghrelin was significantly lower in CED patients than in the controls (156.8 ± 86.7 pg/ml vs. 260,2 ± 87.6 pg/ml, p = 0.0002), their fasting plasma level of PP turned out to be significantly higher (265.2 ± 306.3 pg/ml vs. 54.1 ± 54.6 pg/ml, p = 0.0005) (Fig. 3).

Correlations

An analysis of associations between fasting plasma concentrations of enterohormones and EGG parameters of CED patients showed a significant positive correlation between the level of ghrelin and percentage of time with preprandial bradygastria

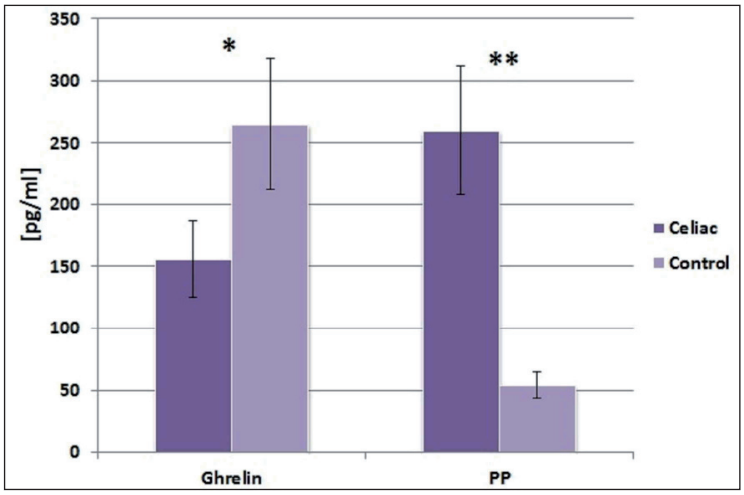

Fig. 3. Comparison of fasting plasma concentrations of ghrelin and PP in patients with celiac disease and healthy controls. * — p = 0.0002, ** — p = 0.0005 — statistically significant intergroup differences.

(r = 0.42, p = 0.049), as well as an inverse correlation between the ghrelin concentration and preprandial DP (r = –0.54, p = 0.049). In turn, fasting plasma level of PP was shown to correlate positively with the percentage of time with postprandial tachygastria (r = 0.42, p = 0.049). Furthermore, duration of the disease was demonstrated to correlate positively with both percentage of time with postprandial tachygastria and postprandial DP (r = 0.52, p = 0.01; r = 0.62, p = 0.03) (Table 3). No significant correlations were found between the levels of inflammatory markers, fasting ghrelin and PP plasma concentrations and EGG parameters.

Table 3. Correlations between the EGG parameters, serum enterohormonal levels and duration of celiac disease.

Parameters	R	P
Ghrelin & preprandial DP	–0.54	0.049
Ghrelin & preprandial % bradygastria	0.42	0.049
Pancreatic polypeptide & % postprandial tachygastria	0.42	0.049
Postprandial DP & duration of celiac disease	0.62	0.03
Postprandial tachygastria & duration of celiac disease	0.52	0.01

DP — dominant power of dominant frequency.

Discussion

To the best of our knowledge, this was the first study to analyze a relationship between the EGG parameters and plasma levels of GI peptides, ghrelin and PP, in patients with CED. The results of this analysis suggest that individuals suffering from this condition present with an autonomic nervous system imbalance.

Several previous studies documented a relationship between EGG parameters and gastric emptying [9, 10]. Delayed gastric emptying was linked to changes in EGG, namely to a decrease in the percentage of normal gastric slow waves and lower postprandial increase in DP. We observed an impairment of EGG parameters in CED patients. A decrease in the percentage of time with normogastria and SWC, with a concomitant increase in log DP, were documented on fasting EGG recordings. Moreover, CED patients did not show an increase in the EGG parameters when re-examined after a standard meal. The abovementioned EGG changes turned out to be associated with a lower fasting plasma concentration of ghrelin. Previous animal and human studies showed that ghrelin enhances gastric motility and gastric emptying, and a decrease in the concentration of this hormone may result in a delayed gastric emptying [15, 20]. Our observation on the preprandial decrease in the percentage of time with normogastria is consistent with the data published by Chen *et al.*, who examined patients with gastroparesis, as well as with the results reported by Kamiya *et al.*, who studied the group of individuals with dyspepsia and non-erosive gastroesophageal disease (NERD) [10, 23]. Since Chen *et al.* documented a decrease in the percentage of time with normogastria and abnormal postprandial DP, it is likely that also our patients presented with a delayed gastric emptying. Similarly, the lack of increase in power ratio observed by these authors likely corresponded to low antral motility and resultant delayed gastric emptying, which also may be extrapolated to our CED group. Furthermore, a positive correlation between a 3-cpm amplitude observed on EGG and plasma PP immunoreactivity level was previously reported by Kaneko *et al.*, pointing to an increase in vagal activity [24]. The above mentioned data are consistent with the results of present study, namely with the fact that our CED patients presented with higher DP than the controls, but showed less pronounced increase in this parameter after feeding. However, a decrease in DP was previously reported by Kamiya *et al.* in patients with NERD and by Cucciara *et al.* in children with acute symptoms of gastroesophageal reflux disease (GERD) [9, 23]. These data support our primary hypothesis that an inflammatory damage to the enteric nervous system (ENS) is a common consequence of a GI inflammation. Chronic inflammation may also result in secondary neuroproliferation in the duodenal mucosa as previously shown by Leonard *et al.* [25]. We suggest that these microscopic changes may interfere with normal neuronal function, but this concept needs to be verified empirically.

The role of sympathetic activation in modulation of gastric slow waves and pathogenesis of CED symptoms have not been established thus far. Dysautonomia may also occur concomitantly to other conditions, such as diabetes mellitus, functional disorders of the GI tract and sclerodermia. According to McNeamey *et al.*, the dysmotility observed in the course of these disorders may result from a primary neuropathic process leading to ENS damage [26]. These authors observed both sympathetic activation and slow wave activity, but did not explain if the abnormalities of the latter occurred secondarily to the autonomic impairment [26]. Our findings are consistent with these data supporting the hypothesis that primary inflammatory damage to the enteric innervation may result in a subsequent decrease in the release of ghrelin [27].

Contrary to our findings, Capristo *et al.* and Peracchi *et al.* observed an increase in the ghrelin levels of untreated CED patients [15, 18], with subsequent normalization of this parameter after switching to a gluten-free diet. Moreover, they demonstrated a correlation between the level of ghrelin and severity of inflammation [17]. This finding was further supported by Cheung *et al.* and Tack *et al.* [12, 15]. The decrease in the concentration of ghrelin observed in our CED patients might be associated with the presence of gastric inflammatory lesions. Zwolinska-Wcislo *et al.* showed that individuals with concomitant CED and lymphocytic gastritis may present with severe dyspeptic symptoms [28]. Therefore, an injury to gastric mucosa may constitute a potential underlying mechanism of inhibited ghrelin release.

Our results are partially consistent with the data published by Capristo *et al.* Although these authors did not find a significant difference in the concentrations of ghrelin of untreated CED patients and healthy controls, they explained this phenomenon as a potential consequence of the disease duration, patient sex and age [18]. Similar to our study, Cummings *et al.* observed a decrease in the ghrelin level of CED patients and interpreted this finding as an effect of autoimmune gastrointestinal disease [29]. The preprandial decrease in the concentration of ghrelin in our CED patients may explain the abnormalities documented on their EGG recordings, being a likely cause for the delayed gastric emptying. The inverse correlation between fasting level of ghrelin and preprandial tachygastria observed in our patients suggests that a decrease in the concentration of this peptide may be a consequence of slow gastric emptying and resultant gastric stasis. This in turn leads to a prolonged food retention in the stomach [12]. The delayed gastric emptying with prolonged food retention in the stomach is known to be associated with the occurrence of dyspeptic symptoms, such as early satiety, nausea and vomiting [12].

CED may cause disturbances in autonomic system activity (ANS). In line with the modes of autonomic control by Berntson [30], we observed a simultaneous coupled non reciprocal co-activation of both the ANS arms. Gastric dysmotility, likely resulting from an autonomic dysfunction, was previously reported by other authors [24, 31]. Due to induced autonomic dysfunction with co-activation of both arms of

the autonomic nervous system, our CED patients showed diminished response to parasympathetic stimulation (as confirmed by the preprandial increase in DP and elevated fasting plasma concentration of PP). We previously showed that CED patients frequently present with dysautonomia in form of the sympathetic overdrive [32]. Also other authors consistently reported sympathetic activation in CED patients [24]. Huda at al. showed that ghrelin inhibits activity of the sympathetic nervous system (SNS) in healthy controls, but exerts only a moderate effect on their parasympathetic nervous system (PNS) [33]. In the abovementioned study, vagotomized subjects did not respond to ghrelin, which suggests that vagus nerve plays an important role in transmitting the effects of peripheral ghrelin to the SNS. Low concentration of ghrelin and concomitant increase in the sympathetic drive point to likely lack of reciprocal SNS inhibition in our patients. Also PNS was postulated to be involved in the induction of ghrelin secretion [34]. While an increase in the circulating ghrelin level in humans was shown to be simulated by cholinergic agonists, cholinergic antagonists were demonstrated to cause a decrease in this parameter. Vagus nerve is known to play an important role in signaling between peripheral ghrelin and central appetite centers. This points to potential involvement of various pathophysiological mechanisms, such as intestinal inflammation and visceral sensitization in food intake control. Our results may have some implications for symptomatic treatment of CED, as modulation of the sympathetic tone may result in changes in gastric motility.

The effects of ghrelin on the activity of SNS and PNS in humans have not been studied in detail to this date. Central nervous system (CNS) communicates directly with the intestine via the brain-gut axis. CNS recognizes GI inflammation present in CED patients as an activation of vagal afferent signaling. Inflammatory signals generated within the bowel may significantly alter peripheral neuronal signaling, which results in both peripheral and central sensitization, a phenomenon that is reflected by an enhanced afferent neuronal activation [35, 36]. As shown recently, the brain not only "detects" peripheral inflammation via the afferent vagal fibers, but also attenuates the innate immune activation due to an integrated neural response involving massive activation of the vagal efferent fibers. This efferent arm of the inflammatory reflex is referred to as the "cholinergic anti-inflammatory pathway". Inflammatory reflex constitutes a crucial homeostatic system. A number of previous studies showed that the release of PP from the pancreas is under vagal control and therefore may be used as a marker of the vagal efferent activity [37]. As direct measurement of the vagal efferent activity is not possible due to rapid degradation of acetylcholine, we used the PP response as an indicator of visceral vagal activity. Such experimental approach may provide an insight into the physiological conditions under which vagal efferent fibers can be evaluated. This hypothesis seems to be strongly supported by clinical data as significantly elevated fasting plasma concentrations of PP were previously demonstrated in both patients with ulcerative colitis and individuals

with Crohn's disease [38, 39]. This suggests that inflammatory bowel disease is likely associated with a shift in the autonomic balance towards a relative parasympathetic predominance, even in the case of remission.

Although Papastamataki and Linnestad observed a different secretion pattern of the gut-brain axis hormones in children with CED, the PP levels of their patients did not differ significantly from those in adults [20, 40]. In contrast to these authors, we found elevated levels of PP in our adult CED patients. This discrepancy may be attributed to presence of different stages of ANS impairment and/or to differences in the immune response of children and adults reflecting a well-known phenomenon of greater ENS neuroplasticity in young persons. As mentioned above, in our previous study we demonstrated sympathetic overdrive in the same group of CED patients [32]. It should be remembered, however, that patients are not homogeneous in terms of their affective states, stress levels and coping strategies, and all these factors may influence their autonomic balance. Furthermore, an increase in vagal efferent activity and/or altered receptor activity may change the sensitivity to acetylcholine as a compensatory mechanism.

As an orexogenic hormone, ghrelin is mainly released in the empty stomach. In some cases, however, gastric distention developed secondarily to impaired motility of the stomach may inhibit the ghrelin release. Moreover, also sympathetic suppression observed in CED patients may impair both ghrelin and PP release.

According to Baatar *et al.*, the expression of endogenous ghrelin may play a homeostatic role in the immunological reactions involved in CED pathogenesis [35]. Probably, ghrelin controls T-cell activity, thus exerting an anti-inflammatory effect, and suppresses tumor necrosis factor alpha (TNF alpha) secretion [35, 36]. In our opinion, lower level of ghrelin may be reflected by persistent inflammatory effusions around the ENS neurons which in turn may lead to either visceral hyperactivity or ANS impairment. This hypothesis needs to be verified empirically, and thus an in-depth research is required on this specific aspect of intestinal immune response [35]. In conclusion, the autonomic dysfunction observed in CED patients may affect their GI motility with resultant impairment of enterohormonal secretion. Aside from well-established role of ANS in the control of GI motility, also efferent vagal nerve stimulation was hypothesized to regulate immune response directly and rapidly. Activation of efferent vagal fibers results in the release of acetylcholine, which not only inhibits the synthesis of pro-inflammatory cytokines but also causes changes in ghrelin and PP release.

Conclusions

Both our findings and the results of previous studies suggest that CED causes disturbances in gastric myoelectric activity and fasting enterohormonal levels as a consequence of inflammatory damage to the ENS. The diminished responsiveness to sympathetic and parasympathetic stimulation points to likely co-activation of both ANS

arms as a common pathophysiological mechanism leading to gastric dysmotility in CED patients. The decrease in plasma concentration of ghrelin and the increase in plasma level of PP may lead to delayed gastric emptying in CED patients. The altered gastric motility may be in turn reflected by presence of dyspeptic symptoms.

The some part of manuscript's content has been presented before as conferences presentation on two Congresses

Title of presentation: "Diminished the upper-gut motility and enterohormons level in the celiac disease patients" on XVI Congress of Polish Gastroenterology Society 25–27.09.2014 in Wrocław, Poland and on 22nd United European Gastroenterology UEG Week 18–22.10.2014 Wien, Austria.

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Conflict of interest

None declared.

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