INTRODUCTION

Celiac disease (CeD) is a complex immune-mediated disorder of the gastrointestinal tract (1). It is characterized by chronic inflammation of the small intestinal mucosa, which may affect the brain-gut axis. The activation of visceral receptors (gastrointestinal mechanoreceptor and osmoreceptor) in response to stomach distension caused by water ingestion has not been studied before. Our results showed reduced responsiveness of the autonomic nervous system to water ingestion in patients with celiac disease, which may lead to disturbances of gastric myoelectrical activity and depends on baseline autonomic activity. Water intake can induce gastric distension and motility response, without changes in gastrointestinal hormones. It can also increase the activity of the autonomic nervous system. On the other hand, inflammation in celiac disease (CeD) can alter visceral perception (increase sensitization), leading to autonomic dysfunction. We aimed to investigate the effect of water ingestion on autonomic activity measured as heart rate variability (HRV) and gastric myoelectrical activity measured by electrogastrography (EGG) in patients with CeD. The study included 53 patients with CeD and 50 healthy controls: mean (SD) age, 43.4 (14.8) years and 44.1 (9.2) years, respectively. Electrocardiography with HRV analysis and simultaneous 4-channel EGG was performed before and after the water load test (WLT) ingestion 500-ml water over 5 minutes. We found that compared with controls, at fasting, patients with CeD showed a reduced percentage of normogastria ($P=0.045$) and an average percentage of slow wave coupling ($P<0.01$) with increased dominant power (DP) ($P<0.001$). Moreover, water ingestion in CeD patients reduced the percentage of gastric arrhythmia ($P<0.01$) and increased the percentage of normogastria ($P<0.01$) and DP ($P<0.01$). Finally, in the CeD group, water ingestion increased HRV indices: low frequency by 116.9% ($P<0.001$), high frequency by 125.3% ($P<0.01$), but they did not reach the values of the control group. Patients with CeD showed a smaller increase in parasympathetic autonomic activity after the WLT than controls. Altered autonomic responsiveness may contribute to the disturbances of gastric myoelectrical activity and depends on baseline autonomic activity.

**Key words:** celiac disease, autonomic nervous system, gastric myoelectrical activity, water load test, electrogastrography, visceral perception, heart rate variability, baroreceptor reflex

EFFECTS OF WATER INGESTION ON AUTONOMIC ACTIVITY AND GASTRIC MOTILITY IN PATIENTS WITH CELIAC DISEASE

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The pathogenesis of celiac disease is associated with an autoimmune process. The disease causes chronic inflammation of the small intestinal mucosa, which may affect the brain-gut axis. The activation of visceral receptors (gastrointestinal mechanoreceptor and osmoreceptor) in response to stomach distension caused by water ingestion has not been studied before. Our results showed reduced responsiveness of the autonomic nervous system to water ingestion in patients with celiac disease, which may lead to disturbances of gastric myoelectrical activity and depends on baseline autonomic activity. Water intake can induce gastric distension and motility response, without changes in gastrointestinal hormones. It can also increase the activity of the autonomic nervous system. On the other hand, inflammation in celiac disease (CeD) can alter visceral perception (increase sensitization), leading to autonomic dysfunction. We aimed to investigate the effect of water ingestion on autonomic activity measured as heart rate variability (HRV) and gastric myoelectrical activity measured by electrogastrography (EGG) in patients with CeD. The study included 53 patients with CeD and 50 healthy controls: mean (SD) age, 43.4 (14.8) years and 44.1 (9.2) years, respectively. Electrocardiography with HRV analysis and simultaneous 4-channel EGG was performed before and after the water load test (WLT) ingestion 500-ml water over 5 minutes. We found that compared with controls, at fasting, patients with CeD showed a reduced percentage of normogastria ($P=0.045$) and an average percentage of slow wave coupling ($P<0.01$) with increased dominant power (DP) ($P<0.001$). Moreover, water ingestion in CeD patients reduced the percentage of gastric arrhythmia ($P<0.01$) and increased the percentage of normogastria ($P<0.01$) and DP ($P<0.01$). Finally, in the CeD group, water ingestion increased HRV indices: low frequency by 116.9% ($P<0.001$), high frequency by 125.3% ($P<0.01$), but they did not reach the values of the control group. Patients with CeD showed a smaller increase in parasympathetic autonomic activity after the WLT than controls. Altered autonomic responsiveness may contribute to the disturbances of gastric myoelectrical activity and depends on baseline autonomic activity.

**Key words:** celiac disease, autonomic nervous system, gastric myoelectrical activity, water load test, electrogastrography, visceral perception, heart rate variability, baroreceptor reflex

INTRODUCTION

Celiac disease (CeD) is a complex immune-mediated disorder of the gastrointestinal tract (1). It is characterized by chronic inflammation of the small intestinal mucosa, which leads to malabsorption. The clinical spectrum is diverse and includes both gastrointestinal and extraintestinal manifestations as well as neurological signs and symptoms (1-4). The disease may cause autonomic nervous system (ANS) impairment, which may manifest itself as an incorrect response of heart rate variability (HRV), blood pressure variability (BPV), or baroreceptor reflex sensitivity (BRS) (5-7). While the causative factors of autonomic dysfunction in CeD are unknown, several investigators suggested the involvement of autoimmune damage, metabolic derangements, malabsorption, or chronic inflammation (8-10).

Previous studies showed that the ANS plays a crucial role in the regulation of cardiovascular reflexes and metabolic events after food ingestion (11-15). Greater gastric distension is associated with relative parasympathetic predominance, but the underlying mechanisms remain unclear (11). Feeding causes stomach distension leading to autonomic response (11). Moreover, it induces cardiovascular response, which is associated with higher sympathetic drive (11-13) and lower blood pressure (BP) due to reduced peripheral vascular resistance (11, 13, 14).

Unlike food ingestion, water intake (water load test (WLT)) evokes gastric motility response without the complex hormonal response of a high-caloric test meal (15, 16). In patients with hypotonia, the administration of water (400–500 ml) leads to an increase in mean BP (16), with sympathetic activation as the underlying mechanism (17). In healthy middle-aged and old-
aged individuals, a WLT was shown to increase BP by 10 mmHg (18). It was also reported that a fast intake of cold water alleviates orthostatic intolerance (19). Gastric myoelectrical activity, assessed by electrogastrography (EGG), can be used to indirectly evaluate the frequency and regularity of the gastric slow wave in numerous gastrointestinal conditions (20-22). It was also reported that gastric myoelectrical activity, which reflects gastric motility, increases after eating and is enhanced during the cephalic phase (23).

Only few studies combined EGG with HRV analysis, which is a noninvasive measurement of autonomic activity, to investigate gastric myoelectrical activity and/or autonomic activity in patients with CeD (24-26) and other gastrointestinal diseases (27-30). They showed that CeD patients in the fasting state had a reduced percentage normal gastric myoelectrical activity, slow wave coupling, and increased dominant power (DP) of the slow wave, as compared with controls. Additionally, gastric myoelectrical response to food was abolished in CeD patients.

Little is known about the correlation between changes in autonomic activity, gastrointestinal motility, and inflammation in CeD. The chronic inflammation process, could cause changes in visceral perception by promoting visceral hypersensitivity. Impaired gastric sensation and accommodation are common abnormalities in CeD patients (5, 31, 32). This type of gluten intolerance is not limited to the small intestine but also affects the nervous system, including the enteric nervous system (affect brain-gut axis regulation), thus leading to visceral sensitivity and altered gastrointestinal motility (33).

The WLT represents a valid tool for assessing gastric signals. In contrast to uncomfortable and unpleasant invasive measures such as the barostat balloon, the WLT is noninvasive, convenient, and participant friendly. Therefore, it seems reasonable to use it as a screening test in patients with CeD to better understand altered stomach perception and symptoms of autonomic neuropathy (5, 32). The analysis of response to stomach distension caused by water ingestion could help identify patients with hypersensitivity. Additionally, the mechanisms of gastrointestinal mechanoreceptor and osmoreceptor activation during the WLT in patients with CeD are unknown. Therefore, the aim of this study was to investigate the effect of water intake (visceral receptor activation) on autonomic activity (measured as HRV) and gastric motility (measured by EGG) in patients with CeD compared with healthy individuals.

**PATIENTS AND METHODS**

**Patients**

The study was approved by the Bioethics Committee of Jagiellonian University in Cracow, Poland (no. of permission, KBET/148/B/2012). All participants were instructed about the aim of the study, and written consent was obtained before enrollment, in accordance with the Declaration of Helsinki.

The study included 103 participants: 53 patients with CeD but without neurological symptoms (13 men, 40 women; mean (SD) age, 43.4 ± 14.8 years) and 50 healthy volunteers without a history of gastrointestinal, neurological, and cardiovascular disorders (12 men, 38 women; mean age, 44.1 ± 9.2 years). Disease severity and adherence to gluten-free diet in patients with CeD were assessed by serological testing for antibodies to tissue transglutaminase (anti-tTG) and by histological evaluation of duodenal biopsies (according to Marsh classification) before enrollment. Anti-tTG titers were assessed using an enzyme-linked immunosorbent assay (Aeskul Diagnostics GmbH, Wendelsheim Germany). Antibody levels above 15 U/ml were considered positive, as per our laboratory standards. All assays were performed according to the manufacturer’s instructions. Of the 53 patients, 10 (19%) were newly diagnosed with CeD. Twenty-two patients (41%) reported adherence to diet, but they had positive serology and/or histopathology results and were thus considered as untreated. The remaining group included 21 treated patients with CeD (40%), that is, patients who adhered to diet and had negative serological and histopathological results. The mean disease duration was 11.7 years in the group of untreated patients and 13 years in the group of treated patients.

Biochemical analyses included the evaluation of serum thyrotropin levels, markers of nutrition (hemoglobin levels, mean corpuscular volume, red blood cell distribution width, lymphocyte count, serum calcium and albumin levels), international normalized ratio, and C-reactive protein levels. The exclusion criteria were as follows: cardiovascular disorders (such as hypertension, coronary artery disease, valvular heart disease, cardiac arrhythmias), neurological disorders, gastrointestinal disorders other than CeD, diabetes mellitus, obesity (body mass index ≥30 kg/m²), tobacco smoking, alcohol abuse, intake of medications that would interfere with gastric myoelectrical and autonomic activity measurements (e.g. β-blockers, α-blockers, and others), history of abdominal surgery, pregnancy, or any chronic disorders that might affect gastrointestinal or autonomic function.

**Gastric myoelectrical activity assessment**

The 30-minute EGG recordings of gastric myoelectrical activity were obtained in the morning after 12-hour fasting and 1 hour after the WLT. All examinations were performed at 8:00 am in stable clinical conditions for at least 48 hours, in a quiet relaxed atmosphere, at neutral room temperature, and with participants lying down. Women were examined in the same phase of the menstrual cycle (follicular), and none of them were examined during menstruation. The recording and analysis were conducted using the 4-channel EGG system (Polygram Net®, Medtronic, Minneapolis, Minnesota, United States). The electrodes of the 4-channel device were placed, and the multichannel analysis was performed according to previous studies (25, 30). The following EGG parameters were assessed: the normal frequency range of gastric myoelectrical activity, normogastria (2.0–4.0 cpm); the low range, bradygastria (1.0–2.0 cpm); the high range, tachygastria (4.0–9.0 cpm); unclassified frequency ranges - arrhythmia (<1.0; >9.0 cpm) - the percentage of arrhythmic electrical stomach activity in a period; the DP share in normogastria in relation to the total power of the whole considered frequency band, dominant frequency, and the percentage of slow wave coupling from a cross-channel analysis, which reflects the coordination of waves between different gastric regions (20-22, 30). An abnormal EGG is defined as the percentage time in 2- to 4-cpm slow waves (normogastria) of less than 70% (21, 22). The ranges were listed according to the instructions of the EGG equipment, and they are consistent with the recommendations regarding EGG analysis.

**Water load test**

For the WLT, still water was used. After a simultaneous 30-minute recording of EGG, electrocardiogram, and BP readings, patients were asked to drink 500 ml of noncaloric water slowly over 5 minutes (100 ml/min). Following the WLT, the 3 parameters were recorded simultaneously for another 60 minutes. The volume of water after which the patient reported stomach fullness was recorded (30).
Assessment of autonomic nervous system activity

The ANS was evaluated on the basis of HRV analysis using the Task Force Monitor 3040 equipment and the Task Force Monitor V2.2. software (CNSystems, Graz, Austria). The frequency-domain analysis of the RR intervals and arterial BP was based on an adaptive autoregressive parameter (AAR) algorithm. The time-domain and nonlinear parameters of HRV were evaluated using the Kubios Premium 3.2.0 software (Kubios Oy, Kuopio City, Finland).

Time-domain heart rate variability indices

The following time-domain parameters were calculated: the standard deviation of all NN intervals (SDNN), which reflects both sympathetic and parasympathetic activity; the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD), which is used to estimate the vagal influence on heart rate; and the number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording divided by the total number of all NN intervals (pNN50), which shows a close correlation with parasympathetic activity (34).

Frequency-domain heart rate variability indices

The following frequency-domain parameters were calculated: 1) power spectral density - total power spectrum (TP) from 0.0033 to 0.4 Hz; 2) very-low frequency (VLF) band (0.0033–0.04 Hz), which reflects the influence of the renin-angiotensin-aldosterone system; 3) low-frequency (LF) band (0.04–0.1 Hz), which reflects the sympathetic component influenced by the oscillatory rhythm of arterial BP and depending on BRS; 4) high-frequency (HF) band (0.15–0.4 Hz), which reflects the parasympathetic component associated with breathing; 5) the ratio of LF to HF components (LF/HF ratio), which reflects the relationship between both components of autonomic activity; 6) normalized LF (LFnu (LF/(TP–VLF)*100)) and normalized HF (HFnu (HF/(TP–VLF)*100)), with the participation throughout the frequency spectrum expressed as a percentage (%) (35, 36).

Frequency-domain blood pressure variability indices

The following frequency-domain BPV indices were assessed: power spectral density - total power of the spectrum; VLF component, which reflects BRV modulated by chemoreceptors of the renin-angiotensin-aldosterone system; LF (<0.1 Hz), which reflects oscillation in efferent sympathetic modulation of vascular resistance dependent on numerous physiological phenomena (it was assessed together with mid-frequency, 0.1 Hz, so-called Mayer waves); HF, which reflects oscillations in respiration; and the LF/HF ratio (37).

The spontaneous BRS was determined by: 1) the sequence method, which detects rising sequences (i.e., an increase in systolic BP (SBP) and longer RR intervals) and falling sequences (i.e., a decrease in SBP and shorter RR intervals), from continuous beat-to-beat time series of RR intervals and SBP recordings; and 2) the baroreceptor effectiveness index, which is the ratio of baroreceptor sequences/events for lags 0, 1, and 2 as related to the number of BP ramps.

Nonlinear heart rate variability indices

The nonlinear HRV indices were as follows: 1) recurrence; 2) determinism; 3) detrended fluctuation analysis (DFA), which quantifies fractal-like correlation properties of the time series and uncovers short-range and long-range correlation: DFAa1 (short-term fractal exponent of detrended fluctuation analysis that corresponds to a period of 4–16 HRV) and DFAa2 (long-term fractal exponent of DFA that corresponds to a period of 16–64 HRV); 4) Poincare plots SD1 and SD2, obtained by a simplified plotting of the N/N+1 values against the NN/n values; the Poincare map represents a reduction of an N-dimensional continuous-time dynamical system to an (N-1)-dimensional map; 5) multiscale entropy analysis, which estimates entropy based on the approximate entropy and sample entropy; 6) Shannon entropy (34, 35, 36).

Statistical analysis

Statistical analyses and data collection were performed using the TIBCO Statistica for Windows, version 13.3 PL (Tibco Software Inc., Palo Alto, CA, USA; Jagiellonian University license). The Shapiro-Wilk test was used to verify the normal distribution of variables, and data were presented as means with SD. The group size was assessed for the applied statistical tests at the significance level of 0.05 and the test power of 0.9. For nonnormal variables (e.g., DP values from EGG), logarithmic transformation (to natural logarithms) was used before further analyses. These variables were presented as medians with minimum and maximum values. For intergroup comparisons of quantitative variables, the unpaired t test (for normally distributed variables) or the Mann-Whitney test (for nonnormally distributed variables) was used. Correlations between the pairs of HRV, BPV, and EGG parameters were assessed using the Spearman coefficients of rank correlation, Wilcoxon signed rank test, or paired t test, depending on data distribution. Significance level was set at a P value of less than 0.05.

RESULTS

All 103 participants had normal thyroid function, and the clinical values of mean corpuscular volume and red blood cell distribution width were within the reference range. Inflammation was excluded based on low C-reactive protein levels (<5 mg/l). One patient had an elevated international normalized ratio of prothrombin time. Two patients had anemia (one patient had microcytosis and the other had macrocytosis).

The demographic and clinical characteristics of participants are presented in Table 1. Patients with CeD had significantly higher mean serum anti-TG levels (<5 mg/l). One patient had an elevated international normalized ratio of prothrombin time. Two patients had anemia (one patient had microcytosis and the other had macrocytosis).

The demographic and clinical characteristics of participants are presented in Table 1. Patients with CeD had significantly higher mean serum anti-TG levels (<5 mg/l). One patient had an elevated international normalized ratio of prothrombin time. Two patients had anemia (one patient had microcytosis and the other had macrocytosis).

Gastric myoelectrical activity

In controls, changes in EGG parameters in response to the WLT were noted, including an increase in the average percentage of SWC (from 62.8% to 63.6%; P=0.04), alongside a decrease in dominant slow-wave frequency (from 3.0 to 2.8 cpm; P=0.04) and the percentage of arrhythmia (from 22.4% to 14.3%; P=0.01) (Table 2, Fig. 1).

Fastig CeD patients showed a reduced percentage of normogastrica (54.3% vs. 63.8%, P=0.045) and average percentage of SWC (52.2% vs. 62.75%, P<0.01) with increased DP (11.3µV2 vs. 10.3 µV2, P<0.001), as compared with controls. Moreover, the WLT decreased the percentage of arrhythmia (28.0% vs. 23.5%; P<0.01) and increased the percentage of normogastrica (54.5% vs. 61.5%; P<0.01) and DP (11.3µV2 vs. 12.0µV2; P<0.001) in CeD patients vs. controls (Table 2, Fig. 1).

Based on definition normal EGG, individuals with CeD presented with EGG abnormalities significantly more often than...
controls. Five controls (9%) showed evidence of EGG abnormalities in either a preprandial or postprandial state. In contrast, EGG abnormalities were observed in 36 patients with CeD (67.9%) examined in a fasting state and in 29 patients (54.7%) examined after water ingestion. The prevalence of abnormal EGG recordings in all CeD patients was significantly higher than in controls both at fasting and after the WLT. Data not shown.

Linear analysis of heart rate and blood pressure variability at fasting and after water ingestion

In controls, stomach tensions induced by the WLT led to an increase in LF (from 836.0 ms² to 1163.4 ms²; \( P<0.001 \)) and HF (from 965.50 ms² to 1224.98 ms²; \( P=0.02 \)) (Table 3, Fig. 2). The following time-domain HRV parameters also increased after the WLT: SDNN (by 21.78%, \( P<0.001 \)), pNN50 (by 10.71%, \( P=0.04 \)), and RMSSD (by 11.21%, \( P<0.01 \)).

In patients with CeD, the spectral-domain HRV parameters in the fasting state were significantly lower than in controls (Table 3). Similar to controls, the WLT induced a significant increase in LF (by 116.89%) and HF (by 125.27%) (Fig. 2). There were significant differences in LF and HF between CeD patients and controls (Table 3).

In the fasting state, the time-domain HRV parameters such as SDNN and RMSSD were higher, but pNN50 was lower, in CeD patients versus controls (Table 3). After the WLT, an increase in LF (by 21.78%, \( P<0.001 \)) and HF (by 25.00%, \( P<0.04 \)) was observed in the CeD group. Patients with CeD were stratified according to the LF/HF-HRV ratio to obtain a frequency-domain measure of

**Table 1.** Demographic and clinical characteristics of patients with celiac disease and controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CeD group (( n=53 ))</th>
<th>Control group (( n=50 ))</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years, mean (SD)</strong></td>
<td>42.4 (15.8)</td>
<td>42.1 (9.2)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Sex, female/male, n (%)</strong></td>
<td>17/8 (68/32)</td>
<td>21/9 (70/30)</td>
<td>0.14/0.21</td>
</tr>
<tr>
<td><strong>CeD duration, years, mean (SD) (range)</strong></td>
<td>8.8 (10.5) (0–40)</td>
<td>0–0</td>
<td>–</td>
</tr>
<tr>
<td><strong>Marsh scale, n</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>23</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Type III</td>
<td>30</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td><strong>Anti-tTG, U/ml</strong></td>
<td>138.5 (80.4)</td>
<td>12.4 (6.2)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Body mass index, kg/m², mean (SD)</strong></td>
<td>21.7 (2.65)</td>
<td>23.8 (0.6)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Albumin, g/dl, mean (SD)</strong></td>
<td>43.6 (5.1)</td>
<td>58.7 (4.2)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>C-reactive protein, mg/l, mean (SD)</strong></td>
<td>2.1 (0.4)</td>
<td>1.2 (0.5)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>White blood cells, 10^9/l, mean (SD)</strong></td>
<td>1.743 (0.849)</td>
<td>2.033 (0.946)</td>
<td>NS</td>
</tr>
</tbody>
</table>

A \( P \) value of less than 0.05 was considered significant. * unpaired \( t \) test; \( \chi^2 \) test.

**Abbreviations:** anti-tTG, antibodies to tissue transglutaminase; BMI, body mass index; CeD, celiac disease; CRP, C-reactive protein; NS, nonsignificant.

**Table 2.** Electrogastrographic parameters in patients with celiac disease and controls before and after the water load test.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fasting state</th>
<th>After water load test</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Celiac disease</td>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SDNN (by 10.14%)</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RMSSD (by 6.72%)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pNN50 (by 11.28%)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LF/HF (by 0.59)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HRV ratio to obtain a frequency-domain measure of</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Celiac disease</td>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SDNN (by 21.78%)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RMSSD (by 25.00%)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pNN50 (by 10.71%)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LF/HF (by 0.59)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HRV ratio to obtain a frequency-domain measure of</td>
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</tbody>
</table>

Data are presented as median (minimum–maximum). A \( P \) value of less than 0.05 was considered significant. * Mann-Whitney test or unpaired \( t \) test; **Abbreviations** bradygastric 1–2 cpm; normogastric 2–4 cpm; tachygastria 4–10 cpm. SWC, slow wave coupling.
parasympathetic-sympathetic balance. The cutoff values for the LF/HF ratio were defined as the mean value of this parameter in controls ±1 SD (34, 38). As a result, we identified 11 CeD patients (20.8%) with an LF/HF ratio of less than 0.6 (evidence of parasympathetic overactivity) and 17 patients (32.1%) with an LF/HF ratio exceeding 1.5 (evidence of sympathetic predominance) in the fasting state. After the WLT, there were 3 patients (5.6%) with an LF/HF ratio of less than 0.6 and 21 patients (39.6%) with an LF/HF ratio exceeding 1.5.

Non-linear analysis of heart rate variability in fasting state and in response to water ingestion

In the fasting state, nonlinear HRV parameters, approximate and sample entropy, were significantly higher in controls than in CeD patients, while recurrency and Shannon entropy were significantly lower in controls. The WLT did not induce significant changes in these parameters in patients with CeD. In controls, changes in response to water intake were noted for the following parameters: Poincare plot SD1 ($p<0.01$), Poincare plot SD2 ($p=0.001$), and DFAα2 ($p=0.04$).

In patients with CeD, water ingestion caused a significant increase only in the Poincare plot SD2 ($p=0.01$). A detailed comparison of HRV parameters between CeD and control groups is shown in Table 4.

Blood pressure variability analysis

At fasting, only LFnu-DBP differed significantly between patients with CeD and controls (47.8% vs. 41.93%, respectively, $p=0.03$). After the WLT, significantly higher LFnu-DBP, LFnu-SBP, and LF/HF-SBP were observed in patients with CeD than in controls.

BRS was higher and baroreceptor effectiveness index was lower in CeD patients than in controls (18.63 vs. 15.9 ms/mmHg; $p>0.05$ and 65.4% vs. 70.79%; $p=0.05$, respectively). After the WLT, a decrease in heart rate (73.4 vs. 66.2 bpm; $p<0.001$) and DBP (73.4 vs. 68.2 mmHg; $p=0.01$) was observed in CeD patients versus controls. Additionally, thoracic fluid content (TFC) after the WLT was significantly higher in controls than in CeD patients (39.8 vs. 32.5 ml/mmHg; $p<0.01$).
Table 3. Comparison of linear (time and spectral) heart rate variability parameters between patients with celiac disease and controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fasting state</th>
<th>After water load test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Celiac disease group</td>
<td>Control</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>81.76 (14.57)</td>
<td>68.12 (5.99)</td>
</tr>
<tr>
<td>pNN50, %</td>
<td>20.91 (2.97)</td>
<td>24.18 (4.64)</td>
</tr>
<tr>
<td>RMSSD, ms</td>
<td>77.31 (19.45)</td>
<td>52.11 (7.13)</td>
</tr>
<tr>
<td>LFnu, %</td>
<td>49.41 (14.31)</td>
<td>48.78 (14.07)</td>
</tr>
<tr>
<td>HFnu, %</td>
<td>50.59 (14.31)</td>
<td>51.23 (14.07)</td>
</tr>
<tr>
<td>VLF, ms&lt;sup&gt;2&lt;/sup&gt;</td>
<td>332.35 (5.42–6344.05)</td>
<td>367.00 (85.47–2305.00)</td>
</tr>
<tr>
<td>LF, ms&lt;sup&gt;2&lt;/sup&gt;</td>
<td>291.93 (1746.46–15900.44)</td>
<td>836.00 (175.73–5544.00)</td>
</tr>
<tr>
<td>HF, ms&lt;sup&gt;2&lt;/sup&gt;</td>
<td>348.5 (2425.97–21947.12)</td>
<td>965.50 (44.19–6927.00)</td>
</tr>
<tr>
<td>TP, ms&lt;sup&gt;2&lt;/sup&gt;</td>
<td>990.90 (1533.39–39662.96)</td>
<td>2440.00 (305.39–14189.00)</td>
</tr>
<tr>
<td>LF/HF-HRV</td>
<td>1.39 (0.93)</td>
<td>0.90 (1.14)</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD) or median (minimum – maximum). A P value of less than 0.05 was considered significant. <sup>a</sup>unpaired t test; <sup>b</sup>Mann-Whitney test.

Abbreviations: SDNN, standard deviation of all NN intervals; RMSSD, the square root of the mean of the squares of differences between adjacent NN intervals; pNN50, the number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording divided by the total number of all NN intervals; TP, total power of the spectrum at 0.0033 to 0.4 Hz; VLF, very-low-frequency; LF, low frequency; HF, high frequency

Other analyses

During the WLT, patients with CeD ingested less water than controls (SD), 422.00 (72.00) ml vs. 500 (0.00) ml; P=0.03. No associations were found between age or sex and the volume of water ingested during the WLT in any of the groups.

We think that the degree of autonomic dysfunction may depend on the severity of celiac disease. In our study we did not analyzing the degree of autonomic dysfunction, but there is the association between the severity of celiac disease (Marsh scale) and autonomic dysfunction. The significant more patients with Type III according to Marsh scale than with Type I had abnormal ANS activity (sympathetic or parasympathetic overactivity) at rest (17 subjects from 30 patients (56.6%) vs. 3 persons from 23 patients (13%); p=0.000, Chi² - test). This issue could be the subject of future research with using, for example, questionnaires to assess the severity of ANS dysfunction.

DISCUSSION

To our knowledge, this is the first comprehensive analysis of associations between HRV, BPV, and BRS parameters with gastric myoelectrical activity in CeD patients in response to the WLT. Our study showed sympathovagal imbalance manifesting as sympathetic overactivity in 32.1% of CeD patients, and evidence of parasympathetic overactivity in 20.8%. These abnormalities correspond with the disturbances of baroreceptor activity at rest. Additionally, the study revealed gastric arrhythmias in CeD patients in the fasting state (67.9% of patients) and after water ingestion (54.7% of patients). Finally, it showed that abnormal EGG recordings in all patients with CeD were significantly more common than in controls, both at fasting and after water ingestion. The presence of CeD resulted in an altered response to ingestion of 500 ml water, as compared with controls.

The finding of sympathetic overactivity in CeD patients, as assessed by HRV and BPV parameters, is consistent with our previous studies (10, 25). Importantly, an altered response to the WLT was shown in approximately three-fourths of patients. Previous research showed that the ANS plays a crucial role in cardiovascular and metabolic regulation following feeding and water intake (11, 12, 39). Importantly, the WLT might be useful to evaluate autonomic activity after stomach distension. Contrary to a caloric meal, water intake evokes gastric motility responses without changes in gastrointestinal hormones (17, 18). In healthy individuals, response to water intake is integrated with cardiac vagal activity and parallel to increased sympathetic vasoconstrictor activity. Roulelidge et al. (39) proved that moderate water volume causes bradycardia but does not lead to an increase in BP, and the pressor response occurs maximally 30 minutes after ingestion (39). Previous studies revealed that plasma volume does not change significantly after water intake (17, 18, 39). Our observations partially confirm those findings because the value of TFC after the WLT was significantly higher in controls than in CeD patients. However, the increase of TFC after water ingestion was not significant in any of the groups. The discrepancies between studies may result from different methods of plasma volume evaluation.
caused by water ingestion might trigger a strong pressor response in patients with CeD. Our observations are consistent with the results of et al. (28) who provided evidence that water drinking, a mechanism that probably reduces the response in healthy persons depends on age and baseline BP (19, 40). A normal response to this stimulus is a 16-mmHg increase in BP, caused by sympathetic activation (17). Our patients with CeD presented with enhanced sympathetic activity; however, they did not show an increased SBP, but rather a reduced DBP. This paradoxical response might result from sympathetic overactivity with the downregulation of adrenergic receptors (41). Jordan et al. (15, 16) reported that in patients with autonomic failure, symptoms of orthostatic hypotension were substantially less severe shortly after water ingestion. The sympathetic activation is a normal response to water drinking, but paradoxically, an associated pressor response is present only in those with autonomic dysfunction and in elderly individuals (16, 39). The pressor response to the WLT in patients with CeD, who presented with significantly lower values of resting HRV indices (LF, HF, and pNN50), caused compensatory vagal activation. The WLT-induced increase in all these parameters was observed mainly in CeD patients. Our observations are consistent with the results of Routledge et al. (28) who provided evidence that water ingestion increases cardiac vagal control, which may counteract the pressure effect of sympathetic activation. The loss of this buffering mechanism explains the pressor response to water drinking, a mechanism that probably reduces the response in patients with CeD.

The altered response to the WLT might be associated with the pathophysiological mechanism described below (Fig. 3). There is evidence to suggest that local changes in osmolality caused by water ingestion might trigger a strong pressor response and contribute to an increase in metabolic rate through sympathetic activation. Hepatic spinalafferent neurons, identified in mice, can detect physiological shifts in blood osmolality through activation of the transient receptor potential vanilloid cation channel 4 (TRPV4) on hepatic and/or portal spinal afferent neurons (41, 42). However, the mechanisms of the osmosensitive hepatic afferent excitation in water drinking-induced sympathetic activation in humans are poorly understood (43).

Previous studies showed that patients with gastrointestinal disease (i.e., functional dyspepsia, active gastric ulcers) in comparison to healthy subjects, presented lower water intake with accompanying dyspeptic symptoms (44-47). Additionally, the WLT facilitated the diagnosis of visceral hypersensitivity in patients with dyspepsia (44). Our results are consistent with those findings because CeD patients showed more evident disturbances in response to the WLT than healthy controls. A significant association between the maximum ingested volume of water and gastrointestinal symptoms, such as nausea and early fullness, was previously demonstrated in patients with functional dyspepsia (46, 47). Similar to our study, there was no significant correlation between age and the maximum volume of ingested water in patients with dyspepsia (49). In contrast, Strid et al. (48) revealed that drinking capacity was associated with sex, with a significantly higher maximal water intake noted in men than in women with functional dyspepsia (48). We found no correlation between sex, age, and the volume of water ingested during the WLT either in controls or patients with CeD. According to literature, male patients with gastroesophageal reflux disease (46) or dyspepsia (47) ingested significantly more water than women.

Table 4. Comparison of nonlinear (fractal) heart rate variability parameters between patients with celiac disease and controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fasting state</th>
<th>After water load test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Celiac disease group</td>
<td>Control</td>
</tr>
<tr>
<td>Poincare plot SD1</td>
<td>31.2 (3.8–689.3)</td>
<td>26.5 (12.0–106.0)</td>
</tr>
<tr>
<td>Poincare plot SD2</td>
<td>74.9 (18.6–796.8)</td>
<td>76.3 (46.9–180.5)</td>
</tr>
<tr>
<td>%REC</td>
<td>40.5 (14.9–85.8)</td>
<td>33.1 (17.6–55.1)</td>
</tr>
<tr>
<td>%DET</td>
<td>98.8 (93.7–101.1)</td>
<td>98.2 (94.99–99.89)</td>
</tr>
<tr>
<td>ShanEn</td>
<td>3.5 (2.4–4.6)</td>
<td>3.2 (2.7–4.7)</td>
</tr>
<tr>
<td>ApEn</td>
<td>1.3 (0.52–1.61)</td>
<td>1.4 (0.76–1.6)</td>
</tr>
<tr>
<td>SampleEn</td>
<td>1.4 (0.45–1.9)</td>
<td>1.6 (0.63–1.9)</td>
</tr>
<tr>
<td>DFAα1</td>
<td>0.91 (0.36–1.53)</td>
<td>1.0 (0.53–1.4)</td>
</tr>
<tr>
<td>DFAα2</td>
<td>0.96 (0.55–1.32)</td>
<td>1.0 (0.63–1.2)</td>
</tr>
</tbody>
</table>

Data are presented as median (minimum–maximum). A P value of less than 0.05 was considered significant. a unpaired t test; bMann-Whitney test.

Abbreviations: %REC, recurrence; %DET, determinism; DFA, the slope of the detrended fluctuation analysis estimated with a linear detrend and with a quadratic detrend, including DFAa1 (short-term fractal exponent of detrended fluctuation analysis that corresponds to a period of 4–16 HRV) and DFAa2 (long-term fractal exponent of DFA that corresponds to a period of 16–64 HRV); Poincare plots SD1 and SD2, obtained by simplified plotting of the values N N n+1 against the values of N N n+1 with accompanying dyspeptic symptoms (44-47). Additionally, the WLT facilitated the diagnosis of visceral hypersensitivity in patients with dyspepsia (44). Our results are consistent with those findings because CeD patients showed more evident disturbances in response to the WLT than healthy controls. A significant association between the maximum ingested volume of water and gastrointestinal symptoms, such as nausea and early fullness, was previously demonstrated in patients with functional dyspepsia (46, 47). Similar to our study, there was no significant correlation between age and the maximum volume of ingested water in patients with dyspepsia (49). In contrast, Strid et al. (48) revealed that drinking capacity was associated with sex, with a significantly higher maximal water intake noted in men than in women with functional dyspepsia (48). We found no correlation between sex, age, and the volume of water ingested during the WLT either in controls or patients with CeD. According to literature, male patients with gastroesophageal reflux disease (46) or dyspepsia (47) ingested significantly more water than women.
women. However, data from healthy volunteers did not confirm these findings.

In our study, we noted that some patients with CeD ingested less than 500 ml of water during the WLT, because they reported early satiation or discomfort, although there were no dyspeptic symptoms. Patients with nausea and general symptoms of early dumping had significantly smaller reservoir capacity, and they were shown to have significantly impaired tolerance to volume loading during the WLT (49). Parkman et al. (50) found that mean water intake in patients with gastroparesis and functional dyspepsia was significantly lower than in healthy controls. The lower water intake during the WLT was associated with more severe symptoms of early satiety and postprandial fullness (50). This is consistent with our findings (49, 50).

The WLT is a standardized test to induce gastric distension and gastric motility response without concomitant complex hormonal response typical for a high-caloric test meal. According to Chen et al. (45), EGG can detect gastric dysrhythmias in patients with an array of various gastrointestinal disorders. Furthermore, all patients with CeD included in our study showed alterations of stomach fullness in response to the WLT (45). They also presented with significantly higher percentages of gastric dysrhythmia time and appeared to be more sensitive to gastric distension during the WLT. This implies that the abnormalities might be caused either by the primary visceral pathology or a more central mechanism, perhaps related to psychological disturbances. However, our study did not provide enough evidence to clarify this.

Our study is limited by the lack of previous studies assessing the effect of the WLT on gastrointestinal and cardiovascular function in patients with CeD. As this is the first study to assess the effect of visceral reception activation on autonomic and gastric myoelectrical activity in patients with CeD, we have no benchmark for our results. We hope that our study will provide the basis for further research on associations between CeD, autonomic activity, and gastrointestinal motility. The second limitation is a limited number of patients, although it was highly selected and sufficient for correct statistical inference.

In conclusion, autonomic activation, particularly the parasympathetic component, after the WLT was reduced in patients with CeD compared with controls. The disturbances of gastric myoelectrical activity in CeD could result from reduced autonomic responsiveness. This complex response to water ingestion (gastrointestinal mechanoreception and osmoreception and/or autonomic activation) might be disrupted by gastrointestinal inflammation, especially in autoimmune process in CeD. Also oxidative imbalance might influence the intestinal inflammation that was discussed by Domas et al. (51).

In summary, our results might help identify patients with hypersensitivity caused by inflammation. As this was a preliminary study, the external validity is limited and further research is needed.

Authors’ contribution: AF: study concept and design, data collection, data analysis, statistical analysis, data interpretation, manuscript preparation; MP-F: study concept and design, study recruitment, data analysis and interpretation, manuscript preparation; KC: data collection; KG: critical revision, data analysis; MZ-W: study concept and design, study recruitment, data interpretation, manuscript preparation.

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