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Alimentary Tract

# The effect of gluten on intestinal fermentation, gastric and gallbladder emptying in healthy volunteers

Michele Di Stefano*, Gabriella Carnevale Maffè, Manuela Bergonzi, Caterina Mengoli, Pietro Formagnana, Antonio Di Sabatino, Gino Roberto Corazza

*1st Department of Internal Medicine, University of Pavia, IRCCS "S. Matteo" Hospital Foundation, Pavia, Italy*

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ABSTRACT

*Background:* The relationship between gluten ingestion and gastrointestinal tract function is a matter of debate.
*Aim:* We analysed the effect of gluten on gastric and gallbladder emptying and intestinal fermentation in healthy volunteers.
*Methods:* Ultrasound measurement of gastric and gallbladder emptying after both gluten-containing and gluten-free meals was performed in 18 volunteers (8 women, age 25.0 ± 2.5 years; BMI 22 ± 1.9). Breath hydrogen excretion after a gluten-containing meal, a gluten-free meal and a gluten-free meal with added gluten powder was measured in 16 volunteers (10 women, age 25.2 ± 2.7 years; BMI 22 ± 1.8). The severity of symptoms was monitored.
*Results:* Gluten presence in the meals was not recognised. Gastric emptying time was 81.6 ± 13.8 min after gluten-containing and 73.9 ± 21.6 min after gluten-free meals ($p = 0.11$). Percentage ejection fraction after gluten-containing meals was 60 ± 9% and 60.6 ± 6% after gluten-free meals ($p = 0.68$). Peak and cumulative hydrogen excretion were significantly higher after gluten-containing than after gluten-free meals (peak: 12.5 ± 7.3 vs 6.5 ± 5.1 parts-per-million, $p < 0.01$; and cumulative: 2319 ± 1720 vs 989 ± 680 parts-per-million/minute, respectively; $p < 0.01$). Adding gluten powder to the gluten-free meal did not modify fermentation. Symptoms were mild and not different after the meals.
*Conclusions:* In healthy volunteers, gluten may induce gastrointestinal alterations. Further studies are needed to clarify which patients could benefit from dietary modification.


## 1. Introduction

Gluten is the most important storage protein of wheat and related grains, such as rye, barley and spelt, and is responsible for the unique baking properties of such cereals, as it determines the water absorption capacity, cohesivity, viscosity and elasticity of dough [1,2]. Gluten is a very complex compound, characterised by a high allelic polymorphism encoding its specific proteins, glutenin and gliadin [3], and by difficult digestion due to its high content of the amino acids proline and glutamine, which are resistant to cleavage by the major human gastrointestinal digestive enzymes [4]. The introduction of gluten occurred late in the Greek and Roman diet and represented an important evolutionary challenge that created the condition for human disease [5]: gluten exposure in genetically susceptible subjects determines coeliac disease (CD), a condition characterised by severe but reversible immuno-mediated lesions of the intestinal mucosa and increased morbidity and mortality [6]. It was shown that gluten could alter intestinal absorption, even in patients with underlying, non-intestinal diseases [7], and in healthy subjects [8].

Gluten ingestion has also been related to a spectrum of extraintestinal diseases, such as ataxia, dermatitis herpetiformis, IgA nephropathy, and recurrent oral ulceration [9,10]. Furthermore, gluten has recently been linked to the non-coeliac gluten sensitivity syndrome (NCGS) [11], whose protean symptoms are believed to improve or disappear after gluten withdrawal, in the absence of mucosal histological changes and serum-specific autoantibodies [12]. As a mechanism, a selective role of the innate immune system has been suggested [13], while other researchers, mainly referring to gastrointestinal symptoms, claimed that ingestion of fermentable oligo-, di-, and mono-saccharides and polyols (FODMAP) played a role [14].

* Corresponding author at: 1st Department of Internal Medicine, University of Pavia, IRCCS "S. Matteo" Hospital Foundation, Piazzale C. Golgi 2, 27100 Pavia, Italy. Tel.: +39 0382 502975; fax: +39 0382 502618.
*E-mail address:* m.distefano@unipv.it (M. Di Stefano).

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However, the potential capacity of gluten to induce intestinal symptoms in non-coeliac individuals is still uncertain and before claiming the existence of a new syndrome or disease it would be advisable to know more about the impact that the administration of gluten has on the intestinal function of healthy subjects. Accordingly, the aim of this research was to study gallbladder motility, gastric emptying and intestinal fermentation after a gluten-containing meal and a gluten-free meal in a group of healthy volunteers (HV).

## 2. Methods

### 2.1. Study design

A group of HV underwent Gastrointestinal Symptom Rating Scale (GSRS) administration, a validated questionnaire to disprove the presence of unknown or underestimated gastrointestinal symptoms [15] and accurate medical history recording to exclude previous and current gastrointestinal disorders, and to confirm the absence of ongoing treatments that may interfere with gastrointestinal function. Then, on separate days at least 10 days apart, they underwent ultrasound measurement of gastric and gallbladder emptying time after a gluten-containing and a gluten-free meal. Moreover, measurement of intestinal gas breath excretion after a gluten-containing meal, a gluten-free meal and after a gluten-free meal to which gluten powder had been added was performed to evaluate intestinal fermentation. All the evaluations were performed in a random order, according to a crossover protocol. The presence and severity of symptoms were monitored during all the tests.

We chose not to perform the ultrasound evaluations after the gluten powder added meal, because this meal would have been easily recognised, leading to imperfect symptom reporting.

### 2.2. Subjects

A group of 45 anti-endomysium antibody-negative HV, members of the medical or paramedical staff or students of the Medical School of University of Pavia, was screened for eligibility. In two cases, reflux-related symptoms were present and the subjects were excluded; six subjects were excluded owing to the presence of Hashimoto's thyroiditis and three to the presence of irritable bowel syndrome. Thirty-four HV were enrolled (17 women, mean age $25.2 \pm 2.8$ years, range 21–30; mean BMI $22 \pm 1.9$ years, range 20–24): 18 subjects were available for gastric and gallbladder emptying study and 16 subjects were available for intestinal fermentation study. None of them had a previous history of intolerance to food sources of FODMAP, nor had they been affected by chronic disorders. All subjects provided written informed consent and the protocol was approved by the local ethics committee.

### 2.3. Gastric and gallbladder emptying study

Gallbladder volumes were calculated using the ellipsoid method [16] using the formula $V = 0.52\,(L \times W \times H)$, where $W$ is the gallbladder width, $H$ is height and $L$ is axial length. All measurements were taken in the morning, after an overnight fast, with subjects in the supine position turned partially on their right side, every 15 min for a total of 120 min. The gallbladder basal volume (BV) was considered the volume in fasting state; the gallbladder residual volume (RV) was considered the smallest volume measured at the completion of the meal-induced gallbladder emptying; the gallbladder ejected volume (EV) was considered the difference between the basal volume and the corresponding residual volume. The gallbladder percentage ejection fraction was considered the difference between fasting volume (ml) and residual volume (ml)/fasting volume (ml) $\times$ 100.

Measurement of the antral area was performed according to Bolondi et al. [17] every 30 minutes for 360 minutes or until the gastric area returned to basal values. All measurements of the gastric antrum were taken in the orthostatic position from the outer profile of the wall because the lumen of the antrum is usually very narrow in fasting patients and the inner side of the wall is too difficult to outline. The cross-sectional area of the gastric antrum corresponding to the sagittal plane passing through the superior mesenteric vein presents an elliptical shape; this area was calculated by measuring the longitudinal ($A$) and the anteroposterior ($B$) diameters using this formula: $\pi AB/4$. Basal and maximum postprandial antral areas were calculated. The total emptying time was identified as the time needed to observe the return to baseline values of the antral area when the tendency line between the antral area and time crossed the basal value; the 50% emptying time (2DT50) was defined as the time when the antral area had decreased to half of its maximum size.

Ultrasound studies were performed by a single experienced operator (GCM), who was blinded to the type of meal consumed.

### 2.4. Breath testing

Breath test was performed in accordance with the Rome Consensus Conference [18]. To avoid prolonged intestinal gas production, due to persisting fermentable material in the colon, the subject consumed a pre-test dinner meal consisting of rice, meat and olive oil [19]. After a 12-hour fasting period, breath testing started between 08.30 and 09.30 a.m., after thorough mouthwashing with 40 ml of 1% chlorhexidine solution [20]. Smoking [21] and physical exercise [22] were not allowed before and throughout the test. Sampling of alveolar air, without deep inspiration and hyperventilation, was performed by means of a commercial device (GasamplerQuintron, Milwaukee, WI, USA) that allows the first 500 ml of dead space air to be separated and discarded while the remaining 700 ml of end-alveolar air are collected in a gas-tight bag. The accuracy of the detector (Model DP12, Quintron Instrument, Milwaukee, WI, USA) was $\pm 2$ ppm with a linear response range between 2 and 150 ppm of $H_2$ and between 2 and 50 ppm of $CH_4$. The presence of an average breath $CH_4$ concentration >5 ppm above that of room air was considered indicative of $CH_4$ production [23].

On three different days, at least ten days apart, subjects underwent $H_2$ and $CH_4$ breath excretion measurement at fasting and after oral administration of three different meals: gluten-containing pasta, gluten-free pasta and gluten-free pasta with the addition of powdered gluten. Air samples were collected under fasting conditions and every 15 min. Breath $H_2$ and $CH_4$ excretion were monitored for a 7-hour period. The area under the time-concentration curve was calculated to estimate cumulative intestinal gas excretion both for $H_2$ and $CH_4$ [24]. Just one healthy volunteer showed negligible $CH_4$ production.

### 2.5. Test meals

Since our fermentation study protocol considered the use of a gluten-free meal with added gluten powder, we had to use different meals for emptying and fermentation studies.

#### 2.5.1. Emptying studies

The test meal was a 95-g serving of gluten-free or gluten-containing pasta, dressed with egg, bacon and cheese (pasta alla carbonara™, Antares, Pavia, Italy). The gluten-containing pasta consisted of 63.5 g carbohydrates, 12.0 g proteins, 5.9 g lipids; the gluten-free pasta consisted of 66 g carbohydrates, 11.6 g proteins, 5.1 g lipids.