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### ***Aspergillus niger* prolyl endopeptidase in celiac disease**

AN-PEP and celiac disease

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#### **Abstract**

We comment here the article by *Stefanolo JP et al* entitled “Effect of *Aspergillus niger* prolyl endopeptidase in patients with celiac disease on a long-term gluten-free diet” and published in *World Journal of Gastroenterology* (2024). Celiac disease is a well recognized, systemic autoimmune disorder. In genetically susceptible people, the most evident damage is located in the small intestine, and are caused and worsened by the ingestion of gluten. For that reason, celiac patients adopt a gluten-free diet (GFD), but it has some limitations, and do not prevent from a re-exposure to gluten. Research aims to develop adjuvant therapies, for example one of the most studied alternative is the supplementation of *Aspergillus niger* prolyl endopeptidase protease (AN-PEP), which is able to degrade the gluten in the stomach, reducing its concentration into the small intestine. The study shows a high adherence to the gluten-free-diet, but **did not address** AN-PEP as a **gluten immunogenic peptides** (GIP) reducer, because it was tested only in patients following a GFD and not in gluten exposing condition. This study therefore opens up new research perspectives in this area and shows that further research is needed to clarify the many points that are still in doubt.

**Key Words:** Celiac disease; *Aspergillus niger* prolyl endoprotease; Gluten immunogenic peptides; Trial; Symptoms; Real-life trial.

Colella M, Cafiero C, Palmirotta R. *Aspergillus niger* prolyl endopeptidase in celiac disease. *World J Gastroenterol* 2024; In press

**Core Tip:** Involuntary or voluntary exposure to gluten in long-term GFD celiac patients could lead to severe and persistent symptoms. *Aspergillus niger* prolyl endopeptidase (AN-PEP) is able to metabolize gluten before it arrives into the small intestine, reducing the CeD-specific symptoms. It is demonstrated by the decrease in the concentration on GIP.

## INTRODUCTION

Celiac disease (CeD) is a systemic autoimmune disorder, in patients with a genetic susceptibility. When one of **these** people introduces gluten with diet, the disease is characterized by the activation of specific T- cells for gluten, because they recognize the specific epitopes. This response can harm the gastrointestinal tissues and cause the characteristic symptoms. Most of the damage is located at the small intestine, and it is caused only by the involuntary or voluntary exposure to gluten. A gluten-free diet (GFD) is recommended in these patients, even if it is a difficult, time-consuming and expensive process, but it represents the only way to avoid the appearance of the symptoms. Indeed, after the exposure to gluten, these patients can experience enteropathy and persistent symptoms due to intestinal mucosal damage. For this reason, there is the necessity to find a new potential therapy, which can be integrated to GFD. Mammalian enzymes cannot degrade gluten, because of its proline and glutamine-rich sequences; the result is that the intestinal mucosa is exposed to gluten immunogenic peptides (GIPs), which is the necessary condition in CeD to reactivate gluten T-cells and the disease. Several probiotics and microbial proteases have been studied, for the purpose of degrading more gluten, but the more efficient at the moment seems to be *Aspergillus niger* prolyl-endopeptidase (AN-PEP) [1-3].

Under optimal conditions *Aspergillus niger* prolyl endoprotease can quickly cleave proteins very efficiently. AN-PEP is active at low pH values, and is resistant to pepsin, so it is able to work in the stomach, reducing the GIP before reaching the small intestine.

This oligopeptidase allows to digest gluten into non-toxic fragments, and its use in production of gluten-free food has been proposed in the last years [3].

For example, in late 2018, Vega K. proposed the GRAS claim for a recombinant strain of *Aspergillus niger* that has shown a high efficacy for the production of gluten-reduced foods, and currently is also used in beer production (<https://www.fda.gov/media/134876/download>).

The study carried out by Stefanolo et al [4] aims to examine the effects of AN-PEP on celiac patients following a GFD, and the potential prevention of the CeD symptoms during an involuntary exposure to gluten. This project was basically an exploratory, placebo-controlled trial. The first phase consists in a 4 wk run-in period, followed by a 4 wk period, in which patients were randomized into two arms: test arm (2 AN-PEP capsule per meal) and control arm (placebo). Stefanolo et al [4] conducted a clinical trial of 8 wk, divided in the first phase of 4 wk of run-in and the second one of 4 wk, in which the patients were randomized into two arms, with different treatment: 2 cp of AN-PEP per meal, and placebo. To check the effects of the trial, the authors assayed the stool gluten immunogenic peptides (GIP), CeD-specific serology, and assessed the Celiac Symptom Index (CSI) and the Quality of Life (QoL) using the SF-36 questionnaire. Only thirty-seven patients were randomized, and 628/640 stool samples were collected. Results show that GIP in 65.6% of total samples (GIP < 0.08 µg/g), was higher than 0.32 µg/g in 0.5%, which can potentially cause a mucosal damage, and lower than the run-in period in 44.7%. This was a pilot, double-blind, prospective, randomized, placebo-controlled study. They aim was to explore if the oral administration of AN-PEP can be useful to prevent the effects of exposure to gluten in CeD patients, but the limited number of participants (only 37 patients actively participated in the study) make it not conclusive and results require to be confirmed in future large cohort studies.

## THE UTILITY OF AN-PEP

Adult patients with CeD on a long-term GFD (more than two years) were enrolled from October 2020 to July 2022. The criteria for enrolling patients were: histological and serological diagnosis of CeD, GFD for more than two years, at least one bowel movement per day, being able to provide serum samples, collecting stool samples every Tuesday and Friday, ability to answer questionnaires. The exclusion criteria were: metabolic disorders, refractory CeD, usage of drugs which increase stool GIP excretion (laxatives, probiotics). A total of 37 patients were enrolled in this study, as they respected all the criteria. The clinical trial was organized in two different phases.

In the first phase, all the patients follow a run-in period, which was important to stabilize dietary adherence. Then, in the second phase, they were randomized in two arms:

17 patients were treated with GliadinX, 2 cp/meal (6 cp/day); 325 mg/cp, made up of 70% AN-PEP (obtained from a genetically modified *Aspergillus niger*), 30% maltodextrin, and citric acid;

the remaining 20 patients were in the control group, which ingested placebo (maltodextrin, citric acid, and microcrystalline cellulose).

Every patient had to collect stool samples every Tuesday and Friday (both in run-in and the second phase), which were subsequently processed with an ELISA kit (iVYLISA GIP-Stool; Biomedal S.L., Sevilla, Spain) to quantify the presence of GIP. The stool samples expected were 640, but only 628 samples arrived and were processed in the laboratory according to current protocols [5-7]. Results show that 65.6 % of all samples (both run-in and treatment period) had undetectable GIP (<0.08 µg/g), indicating effectiveness of GFD, none of these patients had a GIP > 0.64 µg/g. In AN-PEP arm was registered in more than 50% of samples a decrease of GIP, indicating effectiveness of AN-PEP. The statistical analysis consists in the comparison between the two arms with Mann-Whitney or Wilcoxon test (comparison between the beginning and the end of treatment), proportion ( $\chi^2$  test), and the comparison of proportion (McNemar test).

Results showed no statistical significance. The results of statistics were that the comparisons are not significant. However, this study examined only lack of gluten as the experimental condition and did not investigate the condition of exposure to gluten.

Serum samples were assayed for IgA tissue transglutaminase antibodies (tTG IgA ELISA kit, QUANTA Lite™, h-tTG IgA, Inova Diagnostic Inc., San Diego, CA, United States), and IgA antibodies reacting with deaminated gliadin-derived peptides (IgA DGP ELISA kit, QUANTA Lite™ DGP IgA, Inova Diagnostic Inc., San Diego, CA, United States). These assays results showed not significant significant statistical differences between placebo arm and AN-PEP arm.

In addition, questionnaires were distributed to the patients before the randomization and at the end of the trial: the Celiac Symptom Index (CSI), consisting of 36 items about specific and general symptoms affecting CeD patients; and the SF-36 QoL questionnaire, which consists in eight subsections about physical functioning, general and mental health. In each question of CSI, the answer goes from 1 to 5, where 1 means no symptoms and 5 means symptoms with the highest intensity. A lower score was noticed in the AN-PEP arm, compared to the placebo, but the difference was not statistically significant. With regards to SF-36, the score ranges from 0 to 100, where 0 means poorest health and 100 optimal health status. At the end of the study, a better score was noticed in the placebo arm pertaining to general health, and in the AN-PEP arm regarding vitality and the abdominal pain.

According to current literature, AN-PEP seems to have a positive impact on CeD symptoms because it is able to degrade gluten before it reaches the small intestine, so the mucosa is exposed to a lower gluten load [8,9]. However, the results are not concordant and no consensus exists till now about this issue [10,11].

## CONCLUSION

The study did not explore the efficiency of AN-PEP during an exposure to gluten; for this reason, more research is needed to better understand the full potential of it. According to this pilot study, AN-PEP treatment does not significantly decrease the GIP

stool concentration. Although this is a pilot study, the results support further investigation into exploring other adjuvant therapies with GFD.

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SIMILARITY INDEX

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### PRIMARY SOURCES

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**1** Juan Pablo Stefanolo, Verónica Segura, Martina Grizzuti, Abel Heredia et al. " Effect of prolyl endopeptidase in patients with celiac disease on a long-term gluten-free diet ", World Journal of Gastroenterology, 2024

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