Examine.com

Research Digest

Exclusive Sneak Peek

Issue 11, Vol 1 of 2 • September 2015

Click here to purchase ERD

Table of Contents

Gluten-intolerant? There's a pill for that

Randomised clinical study: Aspergillus niger-derived enzyme digests gluten in the stomach of healthy volunteers

Introduction

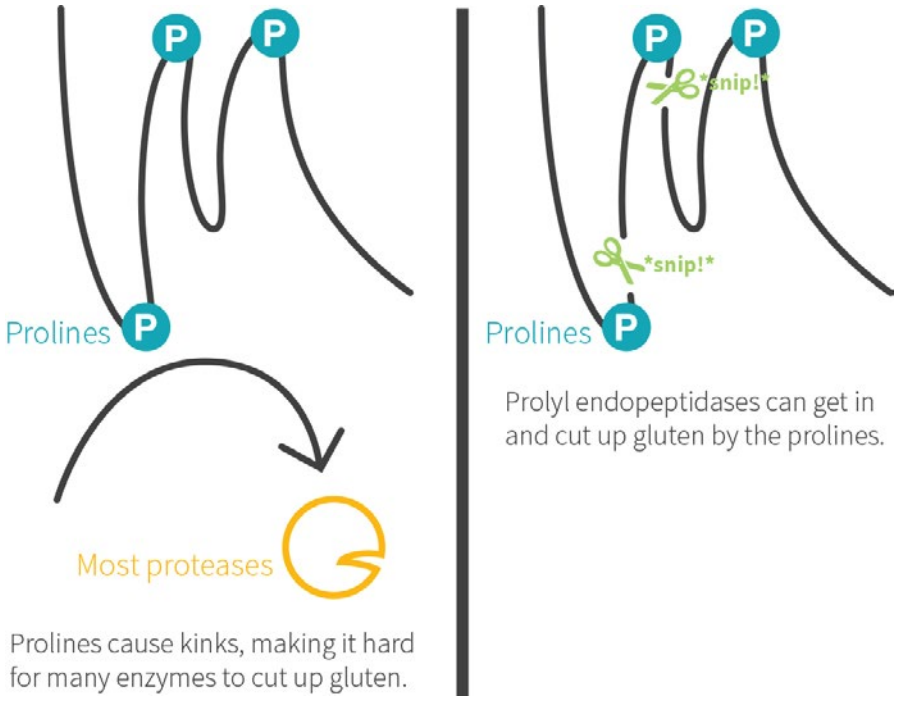
Gluten is a type of protein found in wheat and related grains such as rye and barley, making up about 80% of their total protein content. Normally, proteins are digested in the stomach and upper small intestine (duodenum). However, gluten's structure renders it highly resistant to most of our digestive enzymes, allowing fragments of the gluten protein to persist in the small intestine. More specifically, the gluten protein contains long stretches of proline and glutamine amino acids that require special enzymes to break apart, which humans do not possess. Interestingly, research has identified numerous microbes in both the mouth and colon that can degrade gluten.

It is estimated that at least 1% of the U.S. population suffers from celiac disease, an autoimmune condition characterized by the destruction of the small intestine in response to gluten. Immediate symptoms may include gastrointestinal (GI) distress, headaches, and muscle aches. And long-term gluten consumption can lead to malnutrition, weight loss, and possibly death. The only known treatment option is a lifelong gluten-free diet. However, many foods may contain hidden or unexpected sources of gluten, and food labels on products are not always present. Even items labelled "gluten-free" only need to be below a certain threshold, making them not truly gluten-free. And although non-celiac gluten sensitivity is a controversial diagnosis, research suggests that gluten may damage the guts of people who don't have celiac disease (as explored in ERD issues #7 and #8).

There has been a recent interest in prolyl endopeptidases (PEP, shown in Figure 1), which are a type of enzyme capable of breaking down the proline-glutamine chains within gluten. While early research suggests that PEPs derived from bacteria don't function well due to the stomach's acidity, are rapidly broken down by our own digestive enzymes, and are unable to efficiently prevent the passage of gluten through the intestinal tract, there has been increasing interest of PEPs derived from alternative sources.

In this respect, the *Aspergillus niger*-derived PEP (AN-PEP) has shown promising cell culture results. Additionally, it has proved itself in a digestive model that closely mimics the human GI tract. Most recently, AN-PEP appeared to be well-tolerated in celiac disease patients consuming gluten daily for two weeks, but its efficiency compared to placebo could not be evaluated. The authors of the study under review sought to evaluate how efficiently AN-PEP breaks down gluten in the stomachs of healthy volunteers.

Figure 1: Prolines in gluten make cutting it hard



Gluten is a digestion-resistant protein found in wheat and related cereal grains that can cause extreme distress for people with celiac disease. This study evaluated how efficiently a type of enzyme called AN-PEP breaks down gluten in the stomachs of healthy volunteers.

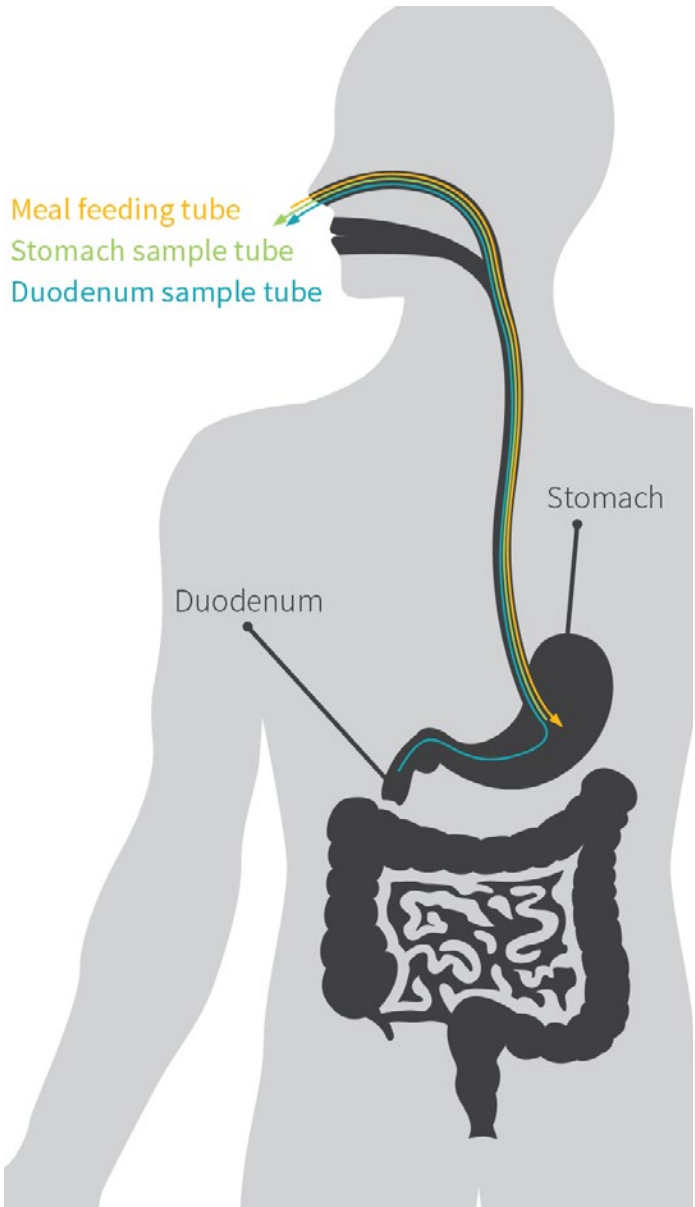
Who and what was studied?

In this double-blind, randomized, placebo-controlled,

crossover study, 12 healthy men and women with no history of gastrointestinal disorders and major diseases underwent four test days with a one-week washout period, where they consumed a high- or low-calorie test meal with AN-PEP or placebo. Each test meal was a powdered mixture of four grams of gluten protein (roughly equivalent to one slice of whole wheat bread), along with added sodium caseinate to balance protein content for the meals, maltodextrin to balance energy content, refined olive oil to add fat content, and acetaminophen to assess gastric emptying rate (through measuring its absorption into the bloodstream). Each group also had either AN-PEP or placebo dissolved in tap water.

The participants didn't actually eat this possibly disgusting concoction. Instead, the "meal" (for lack of a better word) was infused directly into their stomachs by way of a tube going through the nose and into the stomach (shown in Figure 2), along with AN-PEP or placebo. The participants also had other tubes in their stomach and duodenum, where test meal contents could be recovered for analysis.

Figure 2: Down the tube!



This method allowed for the direct measurement of actual gluten content in the GI tract, and allowed for standardized infusion of the test meal and AN-PEP or placebo so as to avoid differences in gluten degradation between interventions from variable meal consumption rates. However, this is obviously not representative of a real meal, where solid food and AN-PEP are ingested separately and undergo the normal physiological processes of mixing in the stomach.

In addition to measuring actual gluten content in the stomach and duodenum through two separate lab procedures, the rate of gastric emptying, and the presence of AN-PEP in samples, the researchers had participants complete a GI symptoms questionnaire.

In this double-blind, randomized, placebo-controlled, crossover study, 12 healthy men and women with no history of gastrointestinal disorders and major diseases consumed a high- or low-calorie test meal containing four grams of gluten (equivalent to the amount in about one slice of wheat bread) with AN-PEP or placebo.

What were the findings?

The results are summarized in Figure 3. Regardless of the caloric content of the meal, AN-PEP ingestion was associated with significantly reduced gluten concentrations in both the stomach and small intestine, compared to the placebo. In fact, gluten concentrations in the duodenum with AN-PEP were so low that they were below the detectable limit for the two lab procedures used (ELISA assay and Western blot).

With the placebo, gluten was detectable within the stomach for three hours after meal consumption,

regardless of caloric content, but significantly less gluten was detectable in the duodenum after the high-calorie meal versus the low-calorie meal. It's possible that the greater fat content of the high-calorie meal increased the secretion of pancreatic enzymes and facilitated gluten degradation. By contrast, gluten was broken down within the stomach in about 60 minutes in both the high- and low-calorie meals when consumed alongside AN-PEP, which consequently led to undetectable amounts of gluten in the duodenum.

AN-PEP itself was detectable only in the stomach, with none found in duodenal samples of any test meal. When food moves from the stomach to the duodenum, it is showered with bile, pancreatic buffers, and enzymes that act to reduce the acidity of the contents. It is therefore possible that under the more neutral conditions of the duodenum, AN-PEP becomes vulnerable to and is degraded by pancreatic enzymes.

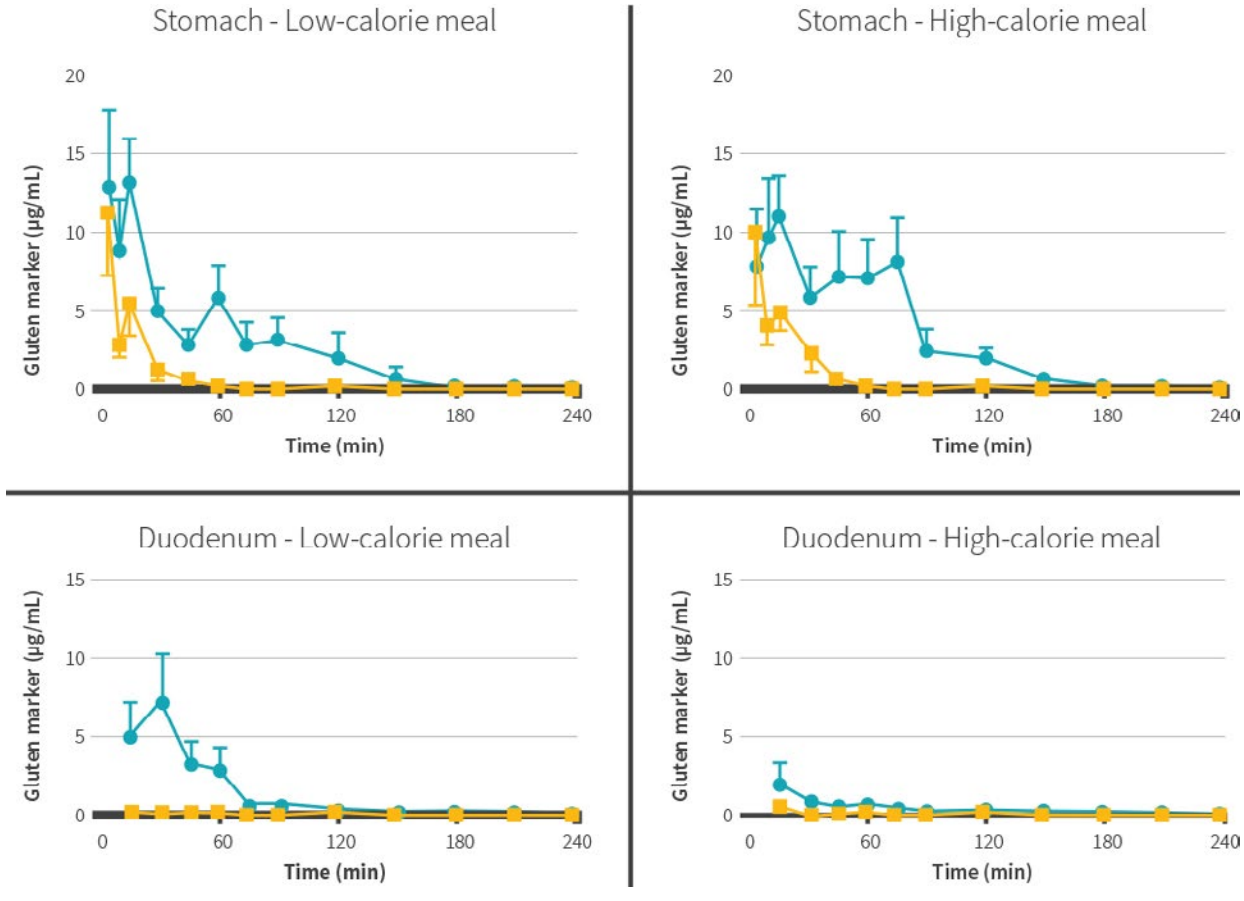
Finally, some mild GI symptoms were reported during the interventions, but there were no differences between AN-PEP and placebo or low- and high-calorie meals.

AN-PEP was able to degrade nearly all the gluten in the stomach within one hour, and then was likely destroyed by the body's own enzymes upon entering the small intestine. It was well tolerated by the participants regardless of the meal's caloric content.

What does the study really tell us?

This study tells us that AN-PEP effectively and safely degrades gluten in the stomach of healthy volunteers. The amount of gluten that entered the stomach was equivalent to about one slice of wheat bread, and the amount reaching the duodenum when ingested alongside AN-PEP was below detectable limits regardless of the caloric content of the meal. Despite these promising results, future research will need to evaluate the effectiveness of AN-PEP in individuals who are most sensitive to gluten – those with celiac disease. It is possible that even the low levels of gluten that entered the duodenum are enough to cause an autoimmune response in this vulnerable population.

Figure 3: Results



Future work will also need to evaluate how AN-PEP interacts with the amount of gluten consumed under more normal conditions, as the current study used a relatively small amount of gluten delivered directly into the stomach alongside AN-PEP, which is not representative of the normal digestive process. It appears unlikely that application of a gluten-degrading enzyme would be within gluten-containing foods themselves, and the most likely supplemental route would be a pill. Since people take pills at various times around a meal and the pill shell takes time to degrade in the stomach, more research is needed to determine how AN-PEP would perform when consumed like most supplements.

Finally, it must be noted that this study was entirely funded by DSM Food Specialties, who currently owns the patent for AN-PEP and have recently introduced it into the American marketplace. Additionally, two of the ten authors are associated with the DSM Biotechnology Center and were responsible for study design and critical revision of the manuscript. No authors declared a conflict of interest.

Despite these limitations, the results are encouraging and suggest that AN-PEP may be a useful adjunct to a gluten-free diet, in order to protect against unintentional and minor intakes of gluten.

Research using gluten-intolerant target populations with normal meal consumption and AN-PEP supplementation patterns will be needed before AN-PEP can be considered safe and effective, but initial results are encouraging and suggest AN-PEP may be a useful adjunct to a gluten-free diet.

The big picture

A gluten-free diet is a necessity for people with celiac disease. More recently, its application has expanded into the treatment of numerous other autoimmune and gastrointestinal diseases with promising results in alleviating symptoms. However, many individuals following a gluten-free diet do not have any of these conditions and yet claim to experience a very similar range of symptoms after eating gluten.

The sea of anecdotal reports eventually spurred clinicians to coin the term non-celiac gluten sensitivity (NCGS), with clinical trials results both supporting the condition and suggesting that it may be overblown. This may be because the mechanism of NCGS remains unknown, making diagnosis reliant upon a recurrence of symptoms when gluten is reintroduced into the diet after removal for a period of time. This is in contrast to celiac disease, for which we have a clear mechanism and mechanism-based diagnostic tools.

In otherwise healthy individuals, gluten consumption has been linked to markers of inflammation, and cell-culture studies have shown gluten to cause increased intestinal permeability, albeit to a lesser extent than in people with celiac disease. Interestingly, having a "leaky gut" is associated with several autoimmune diseases, which may explain why a gluten-free diet has been used successfully to help reduce symptoms of non-celiac autoimmune conditions such as rheumatoid arthritis. However, the overall link between gluten and inflammation in the general population is weak, despite some animal data suggesting a gluten-free diet reduces fat mass, inflammation, and insulin resistance.

Regardless of the true health effects of gluten, some individuals feel better following a gluten-free diet. Whether this is a placebo effect or if they truly suffer from NCGS does not undermine the choice to be gluten-free, since subjective improvement in wellbeing is reason enough to avoid gluten. With that in mind, a pill that may help reduce the likelihood of experiencing gluten's ill effects would be very beneficial.

AN-PEP is not the only gluten-degrading enzyme under

investigation. A barley-derived endoprotease (EP-B2) has been shown to be remarkably effective at digesting gluten in the rat stomach. ALV003, a mixture of EP-B2 and a second complementary protease, has also been shown to be effective in rats, as well as healthy humans. In fact, ALV003 has been shown to prevent biopsy-confirmed small intestinal mucosal injury in patients with celiac disease when consumed alongside two grams of gluten daily for six weeks. Researchers continue to look for bacterial enzymes with gluten-degrading activities.

Gluten is a well-researched compound when it comes to celiac disease, but its role in other conditions, like NCGS, is still being investigated. Regardless of pathology, some individuals may not feel well after eating gluten, which makes having a pill available that could reduce the fallout of accidental gluten consumption invaluable. Clearly the benefits escalate with the degree of harm gluten would cause.

Frequently asked questions

Can gluten be processed out of grains? What are some gluten-free grain sources?

Wheat protein contains about 80% gluten, and because it is bound to the starch within the endosperm of the kernel, processing does not remove it. All wheat varieties contain gluten, as well as relatives such as rye, barley, triticale, malt, brewer's yeast, and basic wheat starch. Additionally, many gluten-free foods, such as oats, are commonly cross-contaminated with gluten because of processing in a facility that also handles wheat-related products.

Ignoring cross-contamination, numerous grains are gluten-free. This includes rice, tapioca, corn, sorghum, quinoa, millet, buckwheat, arrowroot, amaranth, teff, and oats.

What should I know?

Gluten-free diets are contentious: some see them as fads with unsupported claims, and others see them as necessary for optimal gut health regardless of who you are. But there is a subset of the population for which gluten avoidance is absolutely necessary to maximize quality of life.

However, eating gluten-free is not always convenient or possible, putting many individuals at risk for adverse effects. The current study provides encouraging results to suggest that there may soon be an effective enzyme on the market that can successfully break down gluten before it reaches the intestines to cause problems. However, the AN-PEP enzyme isn't likely to be a complete replacement for a gluten-free diet. While it could help offset accidental gluten consumption, its effects as a real-life supplement given with a normal meal need to be further evaluated, in order to fully assess benefits and limitations. ◆

Move over lactase enzyme, a new hot digestive enzyme may be in the limelight soon. Talk it over at the ERD private Facebook forum.

Click here to purchase ERD