

# The role of the gluten-derived peptide gliadin in celiac disease

## Abstract

Gluten is a protein found primarily in wheat that has been associated with celiac disease. Gliadin appears to be the primary cause of celiac disease. Gliadin is a peptide contained within gluten-containing foods, and upon ingestion causes inflammation due to stimulation of helper T-cells. Inflammation is characterized by nutrient malabsorption due to damage of the villi of the mucosal tissue of the small intestine. Celiac disease is strongly associated with the human leukocyte antigens (HLA) DQ2 and DQ8. HLA genes are part of the major histocompatibility complex. Due to the interplay between HLA DQ2 and DQ8 genes, non-HLA associated antigen, and environment, gluten leads to the intestinal damage typical of the disease. The environmental factor that has been known to precipitate the disease is gluten, which can be controlled. On the other hand, genetically speaking, the HLA genes that have been identified to predispose one to the disease are HLA-DQ2 and HLA-DQ8. Celiac disease is a complex genetic disorder, and HLA status appears to be the strongest genetic determinant of risk for celiac autoimmunity. Vitamin B<sub>12</sub> is one of the most common nutrient deficiencies associated with gluten intolerance. The small amount of Vitamin B<sub>12</sub> that does reach the intestine will not be fully absorbed due to the intestinal damage. A subsequent deficiency found in those with celiac disease is that of folate and iron where, in untreated celiac disease, there is loss of brush border proteins and enzymes needed for absorption of dietary folate. Moreover, individuals with untreated celiac disease have been found to excrete fat into their stool because of poor absorption by the small intestine. As a result, they are deficient in omega-6 and omega-3 fatty acids. This is detrimental because these essential fatty acids control inflammation and blood clotting, which is vital in preventing heart disease and neurological disorders.

**Keywords:** celiac disease, gliadin, glutenin, transglutaminase, small intestine, autoimmunity

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**Abbreviations:** HLA, human leukocyte antigens; MHC, major histocompatibility complex; tTG, tissue transglutaminase; TC, transcobalamins

## Introduction

Gluten is a prolamin (gluten protein) found primarily in wheat that has been associated with celiac disease. What is relatively unknown to the general population about gluten is that a certain component of gluten, gliadin appears to be the primary cause of celiac disease. Gliadin is a peptide contained within gluten-containing foods and, upon ingestion, causes inflammation due to stimulation of helper T-cells. It has been estimated that approximately 60,000 annually suffer from celiac disease, suggesting this disease to likely be the common genetically-linked disorder in the United States.<sup>1</sup> The human leukocyte antigens (HLA) DQ2 and DQ8 genes are part of the major histocompatibility complex (MHC), and celiac disease is robustly associated with the up-regulation of these genes. The recognition of T-cells to their respective pathogen relies on the function of MHC molecules to bind peptide fragments for display on the cell surface. The association between celiac disease and HLA is linked to the heightened ability of DQ2 to bind the gluten peptides which have survived deamination by tissue transglutaminase (tTG) during digestion.<sup>2</sup> This T-cell response to HLA-DQ2/DQ8 restricted gluten peptides occurs only in celiac patients and not in healthy individuals.

The HLA-DQ2/DQ8 receptor favors peptides that contain one or more negatively-charged amino acids which are not present in gluten peptides, but can be introduced due to the activity of tTG, which is an enzyme that converts glutamine residues into negatively-charged glutamic acid residues. Gliadin peptides, with their high proline and glutamine content, are ideal substrates for the tTG activity, which is critical for the creation of active T-cell epitopes involved in celiac disease. Several DQ2- and DQ8-restricted T-cell epitopes have been identified, particularly in wheat gluten. An individual is genetically predisposed to celiac disease due to these HLA-DQ2 and DQ8 genes. Gliadin has been shown to cause an inflammatory response in the small intestine of these predisposed individuals that have nutrient deficient consequences, among other things.

## Protein digestion

Protein is an essential part of the diet in maintaining nutrient homeostasis. A normal healthy adult should consume approximately 0.75-0.80 grams per kilogram of body weight in order to maintain nitrogen balance and provide the essential amino acids to the body.<sup>3</sup> Protein digestion in itself is a fairly efficient process that involves a complex series of degradation elicited by the hydrolytic enzymes originating in the stomach, pancreas, and small intestine. Once the protein has begun hydrolysis, a mixture of amino acids and small peptides are rapidly absorbed by the small intestinal enterocytes. In the duodenum, trypsin hydrolytically degrades intact proteins into

single amino acid residues. Upon trypsin-induced up-regulation in chymotrypsin, carboxypeptidase, and elastase, these enzymes are then released into the small intestine for peptide degradation. The majority of ingested protein is assimilated in the jejunum, which has a greater capacity to absorb some amino acids when compared to the ileum. At this point, problems may arise depending on the protein that has been ingested.<sup>4</sup>

A number of diseases can impair protein digestion, absorption, or both, thus resulting in protein-energy malnutrition. One protein that has been shown to cause issues is gluten. Three pathologies are associated with gluten intake: 1) food allergy to wheat, 2) celiac disease, and 3) gluten sensitivity. Of particular concern is celiac disease, an epigenetic-mediated autoimmune disorder of the small intestine manifested as a result of a complex interaction between genetic and environmental factors.<sup>5</sup>

### Gluten: gliadin & glutenin

The grains wheat, rye, and barley contain the structural protein, gluten. Gluten is found in the seed portion of wheat and is made up of gliadin and glutenin, two peptides found in the endosperm of wheat.<sup>6</sup> In bread dough, collectively gliadin and glutenin make a viscous solution. During bread processing, the more kneading of the dough, the more glutinous the dough becomes due to greater cross-linkage bonds formed between gliadin and glutenin. Moreover, the less kneading, the less cross-linkages that are formed; therefore, dough is drier and flakier, thereby resembling more of a piecrust consistency.<sup>4</sup>

During digestion, enzymes are secreted from the stomach, walls of the small intestine, and pancreas that are responsible for degrading intact proteins into single amino acids and di- and tri-peptides. Involved in the digestive proteolysis, when the protein-rich meal reaches the intestines, gluten is degraded by tTG into its constituents, gliadin and glutenin. Glutenin is easily degraded by these digestive enzymes because of its molecular mass which gives it a large surface area for the enzymes to bind, thereby subsequently resulting in degradation.<sup>7</sup> Conversely, gliadin is more resistant to enzymatic degradation due to the fact that this protein is densely-packed which provides it with a low surface area to volume ratio.<sup>8</sup> Gliadin is also abundant in the amino acids, proline and glutamine, which are difficult to digest. Therefore, the consequence of gliadin digestion is the production of oligopeptides, rather than di- and tri-peptides that otherwise occurs with normal protein digestion.<sup>9</sup> The resultant effect of these oligo-peptides formed from gluten digestion is the mediation of an inflammatory response during gluten digestion.<sup>8</sup> This subsequently results in the autoimmune response as antibodies attack gliadin and tTG which then negatively impacts gliadin digestion.

### Celiac disease

In the context of celiac disease, gluten refers to the protein of grains capable of inciting an autoimmune response. Other grains also contain protein, but wheat, barley, rye, and spelt contain varieties that are not broken down by digestive enzymes. In wheat, the protein difficult to digest is gliadin, in rye, it is secalin, and in barley, is hordein. These proteins usually do not affect the gut of the majority of people, but people who have celiac disease react differently to these proteins.

Within the last 50years, the prevalence of gluten intolerance and celiac disease has increased in Western society. A recent study showed that celiac disease has increased from 1 in 650 people to 1 in 120 people over the last 50years.<sup>10</sup> This being said, the wheat eaten today

is not the wheat that was eaten 50years ago. It has been hybridized to illicit faster growing as well as being deamidated to make it water soluble, thus allowing it to be able to be mixed into various packaged foods.<sup>11</sup> This deamidation has been shown to produce a large immune response in many people.<sup>12</sup>

Gluten ingestion is known to propagate an autoimmune response that can be manifested as celiac disease. However, individuals who develop symptoms may not have celiac disease because many simply develop a gluten hyper-sensitivity. As such, gluten sensitivity typically results in gastrointestinal symptoms similar to those in celiac disease, but from the overall clinical perspective, it presents with a lessened severity and is not accompanied by the concurrence of tTG autoantibodies or autoimmune comorbidities that are associated with celiac disease.<sup>6</sup>

### Environmental and genetic factors

Celiac disease is a type of chronic inflammatory disease that is affected by epigenetic (environmental as well as genetic) factors. Due to the interplay between HLA DQ2 and DQ8 genes, non-HLA associated antigen, and environment, gluten leads to the intestinal damage typical of the disease. The environmental factor that has been known to precipitate the disease is gluten, which can be controlled. On the other hand, genetically speaking, the HLA genes that have been identified to predispose one to the disease are HLA-DQ2 and HLA-DQ8. Celiac disease is a genetic disorder with robust complexity, and the strongest genetic determinant of risk for celiac autoimmunity appears to be the individual's HLA status. There is a strong correlating factor for those with celiac disease to carry specific HLA class II alleles, which has been estimated to account for up to 40% of the genetic load. The DQ2 and DQ8 molecules have been shown to present disease-related peptides to T-cells in the small intestine and/or shaping the T-lymphocytes during development in the thymus.<sup>13</sup> There are several distinct populations of T-lymphocytes in celiac disease. The DQ2 T-cells can be isolated and stimulated when cultured with gluten, which induces damage to the normal intestine. Peptide binding studies suggest that the DQ2 and DQ8 molecules have been shown to play a key role in disease pathogenesis in peptide-binding studies, due to their ability to present gliadin to T-cells; therefore, facilitating an inflammatory response.<sup>14</sup>

The also appears to be an involvement of CD4+ T-cells in celiac disease due to the strong association with HLAs. This has been confirmed based on the fact that CD4+ gluten-specific T-cells were isolated from small intestinal biopsies of celiac patients, but not from healthy controls. These findings are noteworthy as they suggest that the DQ2 or DQ8 are important peptide-presenting molecules as they apparently restrict the exclusive activity of T-cells.<sup>15</sup>

### Small intestine inflammatory response

The small intestine of those with celiac disease reacts differently to the aggravating proteins than those without this autoimmune disorder. When these proteins become absorbed into the villous endothelium of the small intestine, an inflammatory response is created which subsequently results in tissue damage. The immune response triggered by the gluten proteins attack the villi, thus causing them to atrophy and erode. As a result, the villi produce fewer digestive enzymes and absorb fewer nutrients. In turn, gastrointestinal distress can occur and is often characterized by bloating, constipation, diarrhea, malabsorption of fat, malnutrition, iron deficiency or anemia, low vitamin D, and possibly even osteoporosis.

In a healthy small intestine, there are millions of fingerlike projections called villi that produce digestive enzymes and absorb nutrients. In people who have celiac disease, the antibodies that are produced to attack dangerous substances also attack gliadin as well as tissue tTG. One of the role of the tTG enzyme is to maintain cellular cohesion of the microvilli in the gut, so when it is attacked, the microvilli degrade. It has recently shown that tTG causes selective deamidation of gluten proteins, which increases their stimulating effect on gluten-sensitive T-cells obtained from the small intestine of patients with celiac disease.<sup>16</sup>

### Zonulin

In celiac disease, the endothelial cells of the small intestine release zonulin in response to gluten. More specifically, zonulin levels are elevated upon exposure to gliadin regardless of whether or not an individual has celiac disease. Zonulin is a protein associated with proteolysis and subsequent degradation of the tight junctions of the enterocytes which ligates the small intestine. As a result, the integrity between tight junctions diminish subsequently allowing the indiscriminate passage of toxins, microbes, undigested food particles, and antibodies to escape into the circulation. Of these escapees, the antibodies are those initially produced with the responsibility of attacking gliadin. As a result, this cascading immunological activation results in augmented damage to the enterocytes, thereby causing elevated inflammation and gut permeability. As the damage progresses, the absorption capability of the microvilli becomes compromised, which can lead to various nutrient deficiencies. It should be mentioned that a previous study has shown that gliadin-induced elevations in zonulin release can also occur in people without the gene for celiac disease. In this study the researchers concluded that intestinal permeability can be associated with up-regulated zonulin signaling in response to gliadin, regardless of the genetic expression of autoimmunity.<sup>17</sup>

### Nutrient malabsorption

Since the small intestine is responsible for absorbing nutrients, damage to the intestine can hinder its ability to absorb nutrients and cause nutrient deficiencies. Vitamin B<sub>12</sub> is one of the most common nutrient deficiencies associated with gluten intolerance. There is less of this vitamin reaching the intestine because the specific transport proteins, transcobalamins I (TC I) and II (TC II), necessary are not being produced as quickly. The small amount of Vitamin B<sub>12</sub> that does reach the intestine will not be fully absorbed due to the intestinal damage. Because the intestine cannot absorb nutrients to the fullest extent, there is also less fat absorbed, which creates a problem for fat-soluble vitamins since they rely on the absorption of fat for their own absorption in the intestine. The fat-soluble vitamin, Vitamin D, is vital to maintaining bone health and regulating other bodily functions. This vitamin is required for the absorption of calcium, so lacking it can lead to osteoporosis.

A subsequent deficiency found in those with celiac disease is that of folate and iron. Folate absorption takes place predominantly in the proximal small intestine where, in untreated celiac disease, there is loss of brush border proteins and enzymes needed for absorption of dietary folate.<sup>18,19</sup> Iron deficiency is a multifactorial process and is characterized by nutrient malabsorption of the mucosal cells, damage to the brush border membrane, rapid turnover of epithelial cells secondary to iron loss, and occult intestinal blood loss.<sup>20</sup> With celiac disease, vitamin B<sub>12</sub> deficiency appears to be highly associated with malabsorptive issues with ileal mucosa; however, deficiencies

of specific transport proteins may also be involved.<sup>21</sup> Vitamin B<sub>12</sub> deficiency causes diarrhea and/or constipation, fatigue, and loss of appetite, and can lead to more serious neurological symptoms.

Moreover, individuals with untreated celiac disease have been found to excrete fat into their stool because of poor absorption by the small intestine. As a result, they are deficient in omega-6 and omega-3 fatty acids. This is detrimental because these essential fatty acids control inflammation and blood clotting, which is vital in preventing heart disease. A study was done to investigate the profile of serum fatty acids in newly detected celiac disease patients before and after treatment with a gluten free diet. They found that there was an abnormality in the fatty acid profile of adult patients with celiac disease, predisposing to a risk for long-chain fatty acid deficiency. Long chain polyunsaturated fatty acids are important to normal metabolism, thus being deficient in this can lead to various health problems such as dermatitis as well as neurological disorders.<sup>22</sup>

### Conclusion

The ability of the small intestine to properly absorb nutrients can be blunted by the presence of gliadin, a prolamin gluten protein found in wheat-containing foods and those from other common grains such as barley and rye.<sup>23</sup> As a result, this situation is often attributed to celiac disease, which is an autoimmune disorder that is manifested in the small intestine. The tTG enzyme modifies the gliadin in a manner in which the mucosal cells of the small intestine become inflamed. This results in degradation of the endothelial cells, thereby negatively impacting to a point at which they atrophy. Subsequently, this consequence results in nutrient malabsorption. Indeed, celiac disease results in sensitivity to gliadin; however, it should not be confused with wheat allergy which may often present itself with similar symptomatology. In regard to effective treatment of celiac disease, presently a prudent diet which is lacking in gluten is the primary recommendation.<sup>24</sup> Strict adherence to a gluten-free diet can minimize irritation and degradation of the endothelial cells within the small intestine. In so doing, this can facilitate healing to the small intestinal mucosa, and may resolve most, if not all, symptoms depending on how recent the diet is begun once the disease has been identified. Adopting a prudent gluten-free diet can help attenuate the elevated risk of osteoporosis and intestinal cancer.<sup>25</sup>

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### Conflict of interest

The author declares no conflict of interest.

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