THE EFFECT OF WHEAT EVOLUTION ON CELIAC DISEASE PREVALENCE

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ABSTRACT

Research has revealed that the genetic makeup of modern humans is nearly identical to that of our *Homo sapien* ancestors from 35,000 to 20,000 years ago. But while our genetics have remained constant since Paleolithic times, our way of living, and most particularly our way of eating, has not. With the advent of agriculture only 10,000 years ago, humans began cultivating cereal grains such as wheat, which were essentially absent from the original human diet. Recent analyses have revealed a correlation between this introduction of wheat and the increased prevalence of grain-related allergies and intolerances, most particularly celiac disease. The purpose of this project is to trace the history and role of wheat in the human diet, paying specific attention to how the introduction and selective breeding of this dietary staple may be connected to human health and disease risk. Through an examination of the altered genetics of modern wheat cultivars as well as an examination of changes in the prevalence of celiac disease across wheat-based societies, it is hypothesized that the human-induced changes to wheat genetics has played as much of a role in the rise of celiac disease as human genetics.
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INTRODUCTION

Along with rice and maize, wheat exists as one of the most commonly consumed dietary staples in the world. Since its introduction during the agricultural revolution, this cereal grain has provided a significant source of both protein and carbohydrate to over half of the world’s population.\(^1\) But in addition to its common dietary status, wheat is also related to one of the most common genetic disorders: celiac disease. Celiac disease is an autoimmune condition characterized by an inflammatory response to the wheat protein gluten. Although reported cases of celiac disease go as far back as the first century AD, increases in the condition’s prevalence have only been noted in recent decades. To some extent, heightened awareness and improved diagnostic tools have been responsible for greater disease diagnosis, but recent studies have revealed that these factors alone are not enough to explain the substantial increases worldwide.

To understand the changing prevalence of celiac disease it is first important to recognize that the development of the condition is dependent upon two necessary components, one genetic and the other environmental. On a genetic level, celiac disease involves the expression of specific alleles responsible for recognizing gluten as a foreign protein. These genetic factors, or human leukocyte antigens (HLA-DQ8 or HLA-DQ2), exist in all cases of celiac disease and are responsible for the disorder’s immune response. But even prior to this genetic response, there must first be an environmental component, and that is the presence of gluten in the diet.

Based on the Paleolithic theory, it has been determined that human genetics have changed relatively little since the introduction of agriculture about 10,000 years ago. Given this relatively constant genome, an argument can be made that any recent increases in
disease prevalence are more likely to be the result of environmental, rather than genetic factors. Through a historical analysis, it appears that one of the most substantial environmental changes related to the human diet has been in regard to wheat. With the introduction of agriculture, cereal grains such as wheat, which had no previous significance in the human diet, suddenly became ubiquitous staples. Over time, these staples were not merely grown, but were heavily selected for certain desirable characteristics such as free-threshing and superior gluten content. And as a result, the composition of modern wheat has been drastically altered.

The aim of this paper will be to examine the environmental influence of wheat within the diet and how changes to this common dietary component may be connected to celiac disease prevalence. To begin, there will be a discussion of the Paleolithic lifestyle and how the advent of agriculture has drastically changed human eating patterns. These sudden dietary changes will then be connected to the genetic basis of celiac disease, examining how this condition may have originated and how its prevalence has been altered over time. Finally, attention will be focused on the historical domestication and selective breeding of wheat, analyzing how the human-induced manipulation of this global dietary staple might be connected to increasing celiac disease rates.
PALEOLITHIC BEGINNINGS: ANCIENT GENES AND NEW GRAINS

PALEOLITHIC BACKGROUND

Natural selection is the process by which environmental pressures change species’ genetic makeup over time. Such a process, however, is by no means immediate. In terms of early human evolution, natural selection was essential in carefully tailoring the human genome over millions of years to the vast variety of biocultural influences the species was subjected to. Since the advent of the agricultural revolution only 10,000 years ago, however, such genetic change has been extremely minute. With the introduction of farming quickly followed by the Industrial Revolution, cultural change has far outpaced the rate of genetic adaptation. Analyses have shown that although lifestyles for modern humans have changed dramatically over the past few centuries, our biochemistry, and thus nutritional needs, are nearly identical to that of our Paleolithic ancestors from 35,000 to 20,000 years ago.² And thus, there exists a great discordance between our ancient genetics and our modern lifestyle.

It has been estimated that the late Paleolithic era was the last time the human gene pool interacted with the type of environment it had been originally selected for.² During this time, the diet of hunter-gathers, our genetic equivalent, was heavily dependent on plants and wild game of minimal or no processing. Nutritionally speaking, the diet was exceptionally wholesome: high in lean protein and fiber, low in simple sugar and sodium, and abundant in micronutrients. In many aspects, the Paleolithic eating pattern differed immensely from that of contemporary times, especially in regard to carbohydrate consumption. Although overall carbohydrate intake of Paleolithic times was nearly equal to that of modern man, the sourcing of this macronutrient was vastly different. Today, carbohydrates make up approximately 50% of the diet, nearly 85% of which are sourced from highly-refined cereal
grains. By contrast, for the hunter-gatherer, 65-70% of overall intake was provided by not just one food, but rather a vast variety of uncultivated fruits, roots, nuts, and legumes, and cereal grains, our modern staple, were virtually absent. These wild plant foods were not only naturally low in glycemic index and sodium but also exceptionally high in phytochemicals and micronutrients. As a result, the diet of hunter-gatherers easily surpassed any current vitamin and mineral recommendations, a situation which today is nearly impossible without the use of supplementation.

It is evident that with such a varied and nutrient-dense selection of food, the overall nutritional status of our hunter-gather ancestors far exceeded that of modern humans. But such superior health was short-lived, for with the revolutionary introduction of agriculture came not only a drastic change in lifestyle, but also a rapid decrease in human nutritional health.

**ADVENT OF AGRICULTURE**

Despite its incredibly sudden onset, agriculture has been considered the greatest of all human revolutions, for both its cultural and its biological implications. First and foremost, agriculture was a dynamic force in creating modern civilization as we know it today. Before the introduction of crops, human populations were constantly moving in order to find adequate food sources. Foraging took a great deal of time and energy, and the payoff was never predictable. The domestication of crops was thus an invaluable addition as it represented greater stability, predictability, and control to a population that was dependent on the undependable. With movement no longer a necessity, humans began to gain a sense of ownership to the land and settle in communities, forming the first stable villages. And
with the time saved by farming, new effort could be focused on developing societal
structures and technological developments. In this way, agriculture opened the door to both
modern technology and a more fully developed human civilization.

The cradle of civilization is attributed to the Middle East because it is within this
geographic area that the beginnings of agriculture first appeared. It is believed that the first
cereal crops, which included barley, peas, lentils, and wheat, were grown in a small area of
the Fertile Crescent between southeastern Turkey and northern Syria nearly 12,000 years
ago. Although these new cereal crops had previously been present in only minute amounts
in the human diet, they nevertheless became an impetus for a revolution that spread across
the globe, from China to the Americas. One reason for this rapid adoption of agriculture was
the change in food availability. The end of the Paleolithic era 10,000 years ago was met by
a widespread extinction of large animals throughout Europe, Asia, and North America. In
light of these events, hunter-gatherers were forced to shift their reliance from larger game to
a mixture of small mammals, fish, and plants to meet their daily caloric needs. But when the
pressures of a burgeoning human population put even these food sources in danger of
depletion, the new cereal crops were adopted as viable means of supplying both energy and
protein.

Although it is true that agriculture has certainly had great cultural influence, it is its
nutritional consequences that have perhaps been even more noteworthy. The adoption of
cereal grains as a significant portion of the human diet brought about a marked decrease in
diversity of the diet, most particularly in regard to plants. With the introduction of farming,
humans changed from a diet of over 100 different species of animals and plants to one
dependent on a few starchy crops. In the post-agricultural diet, vegetable and fruit
consumption dropped to a mere 23% of overall intake, less than a third of what was originally consumed by hunter-gatherers. Today, a mere 17 species make up nearly 90% of the modern human food supply, the vast majority of which are cereal grains. As the main Middle Eastern crop, wheat in particular has become one of the most important staples of human consumption, making up 29-30% of all cereal production and supplying a main source of protein and over half of carbohydrate intake for the majority of the world. Indeed, it is estimated that bread supplies nearly 50 percent of total caloric intake for over half of the globe. Within a few centuries, a crop which had had no previous evolutionary presence suddenly became the ubiquitous staple. And nutritional status has changed drastically as a result.

The great emphasis on fruits and vegetables in the Paleolithic diet meant that hunter-gatherers received a significant amount of fiber, vitamins, and minerals in their daily diet. By contrast, wheat, even in its whole grain form, is wholly lacking in essential micronutrients such as calcium, iron, zinc, omega-3 fatty acids, and vitamins A, C, and B₁₂. Thus when wheat took over as the primary caloric contributor, it displaced much more nutrient-rich sources, causing a nutritional imbalance and increased risk of nutrient deficiency. What is more, domestication of wheat has further devalued the grain’s nutritional content. Through a comparison of cultivated and non-cultivated cereal grains, it has been revealed that domesticated grains have a much higher starch-to-protein ratio as well as a typically higher glycemic index than their wild alternatives. And because most of these grain products are eaten in the form of highly refined flours, any fiber and micronutrients that are present in the bran are lost through its consequential processing.
This shift from a diet rich in fiber and essential micronutrients to one lacking in essential fatty acids, vitamins, minerals, and phytochemicals has been connected in many ways to a decline in overall human health. Examination of human remains just before and just after the advent of agriculture, for example, have revealed that the adoption of grains lower in zinc and iron is connected to an increased incidence anemia-related bone lesions as well as a greater susceptibility to bacterial infection. Furthermore, the switch from diet abundant in animal meat to one founded on lower-protein wheat was met by a decrease in stature by an average of six inches along with an increase in osteomalacia and dental caries of those born after the introduction of farming. Clearly, when comprising such a large portion of the diet, cereal grains prove to be a less optimal nutritional choice. But even on a more basic level, they may be a poor genetic choice. When looking at the digestive processes at work, it can be seen that humans are unable to fully process certain elements of wheat, a problem that may be rooted in our ancient genes.
CELIAC DISEASE: ORIGINS AND CHANGING PREVALENCE

GLUTEN METABOLISM

The most problematic aspect of wheat exists in one of its most characteristic properties: gluten. Gluten, which is composed of glutenin and gliadin subunits, is the main storage protein of wheat. It is also an essential aspect of modern food production, contributing the critical elasticity and texture to bread and other baked products. Recent research, however, has shown that although essential in baking, it is this protein that is of particular issue in terms of human digestion.

In normal digestion, protein catabolism first begins in the stomach, where proteins are cleaved into smaller polypeptides and protoses by the enzyme pepsin. The vast majority of protein digestion, however, occurs in the small intestine. As the undigested food, or chyme, enters the upper portion of the bowel, pancreatic enzymes and peptidases along the brush border are released to breakdown any remaining polypeptide chains into smaller di- or tripeptides capable of transport across the intestinal lining. The protein gluten, however, is especially unique in that it is inherently resistant to all of these enzymatic processes. Studies have shown that the gliadin portion of gluten is particularly rich in proline and glutamine residues, which require propyl endopeptidase activity for cleavage. Gastric, pancreatic, and brush border membrane enzymes, however, characteristically lack such essential activity, and as a result, intact gliadin ogliopeptides, sometimes up to 50 amino acids long, accumulate in the intestine.
GLUTEN METABOLISM IN CELIAC DISEASE

While for most of the population this incomplete digestion is not an issue, in a growing number of individuals with the human leukocyte antigen HLA-DQ2 or HLA-DQ8 gene, the presence of gliadin oligopeptides causes a very distinct autoimmune response known as celiac disease.\(^\text{11}\)

In the human body, HLA molecules are essential to the immune system, functioning as both self- and foreign protein recognition sites. Present on platelets, lymphocytes, macrophages, and monocytes, these molecules exhibit specific peptide-binding grooves that attach to foreign antigens and present their toxic epitopes to T-lymphocytes. When T-cells recognize these epitopes, they bind to the peptide-HLA complex and initiate the production of lymphokines, a class of immune cell, and the synthesis of immunoglobulin antibodies intended to destroy the foreign bodies.\(^\text{13,14}\) For all HLA molecules, this binding to foreign peptides is highly specific and requires only a short peptide segment to initiate an immune response. Like a lock and key, binding occurs when the peptide exhibits a specific residue that matches the groove of the HLA complex.\(^\text{13}\) And in terms of celiac disease, this residue exists in the gliadin portion of gluten.

The HLA-DQ2 and HLA-DQ8 molecules that are characteristic of celiac disease exist on the dendritic cells of the small intestine, functioning to protect the body from any foreign substances that may pass from the GI tract into the body. When intact gluten peptides cross the brush border membrane into the subepithelial region of the small intestine, these proteins exhibit a particularly high affinity to tissue transglutaminase (TGase). TGase, an enzyme used in tissue repair, is unique from gastric, pancreatic, and brush border enzymes in that it is able to deamidate glutamine into negatively-charged
glutamic acid. In this deamidation, however, the toxic T-stimulatory regions, or epitopes, of glutamine-rich gliadin are exposed and bind to HLA-DQ2 or HLA-DQ8 molecules.\textsuperscript{15} This binding triggers the proliferation of T lymphocytes and cytokines, which produce an inflammatory response aimed at attacking not only the bound gliadin peptides, but the intestinal mucosa as well. As a result, the villi of the proximal and middle sections of the bowel are flattened, compromising normal secretory, digestive, and absorptive functions of individuals exhibiting celiac disease.\textsuperscript{16}

Although there are a large number of peptides that can be formed as a result of gluten metabolism, only a select number of these sequences can actually be disease-causing. Based on the target sites necessary for TGase cleavage as well as the sequences needed for HLA-DQ binding, approximately 50 gluten peptide sequences from the $\alpha$- and $\gamma$-gliadins of gluten have been identified as having the potential to stimulate a T-cell reaction.\textsuperscript{15} It has additionally been proposed that the longest of these gluten peptides, those with the most HLA-DQ binding epitopes, have the greatest T-stimulatory potential. For this reason, research has focused on the 33-amino-acid peptide originated from $\alpha$-gliadin, which is believed to be the immunodominant gluten peptide.\textsuperscript{11} And such appears to be true in that $\alpha$-gliadin has been known to trigger an immune response in nearly all cases of celiac disease.\textsuperscript{17}

Celiac disease, which appears to have originated from the innate inability of humans to properly break down gluten, has recently become one of the most common genetically-based diseases. Current estimates place the prevalence of the condition at 0.5-1% of the general population, but due to the fact for every one diagnosis, five to ten cases go undiagnosed, this rate may be even higher.\textsuperscript{18} Such statistics are important to note considering the numerous consequences that arise when the condition is left untreated.
Research has revealed that untreated celiac disease is present in 9-10% of cases of childhood idiopathic short stature and 1.5-7.5% of patients with rheumatoid arthritis while additionally being connected to conditions such as osteoporosis, neurologic problems, infertility, and hepatitis. Due to the severity of all these complications, it is of paramount interest that more effort is placed into understanding exactly how and why celiac disease occurs.

A further problem exists in the fact that worldwide, the prevalence of celiac disease appears to be rapidly increasing, especially across Europe and the United States. Although studies of the pathophysiology of celiac disease certainly support the condition’s genetic basis, they do not fully explain the changing disease trend. For while possession of the HLA-DQ alleles is necessary for celiac disease, these alleles alone are not sufficient for development of the disorder. As it turns out, the gene for HLA-DQ2 or HLA-D8 is present in at least 40% of the adult US population, but 39% of these cases are disease-free. This discrepancy suggests that the development of celiac disease is not purely genetic; there must be an environmental component involved.

**CELIAC DISEASE BEGINNINGS**

The condition of celiac disease certainly has one extremely influential environmental factor, and that is gluten. The connection between celiac disease and gluten was first realized in the 1930’s by Willem-Karel Dicke, a clinician at Juliana Children’s Hospital in the Netherlands. At this time, the symptoms of celiac disease were well-known to medical practitioners, but the cause and proper treatment needed were not. Without an understanding of the condition, most professionals recommended rest and a completely fruit-based diet to their patients. Dicke, however, was revolutionary in that he was one of the first individuals...
to suspect that wheat might be to blame for the little-understood condition. In 1936, Dicke began conducting wheat-free dietary trials on many of his young patients and noted great improvement in their symptoms, growth, and weight. The presence of World War II only further confirmed this notion when rationing greatly restricted the amount of bread products available to Dutch hospital patients. During this period of unintentional wheat-restriction, the symptoms of Dicke’s young patients improved dramatically, thereby supporting his claim that wheat was, in fact, the offending agent of celiac disease.19

**DIAGNOSING THE PROBLEM**

Diagnosis has always been somewhat of a problem in regard to celiac disease in that the symptoms are not only highly various, but also extremely vague. Even the supposed “classic” signs of the disease such as diarrhea, bloating, mineral deficiency, weight loss, and failure to thrive are common to many other clinical conditions and therefore often misattributed. Until Willem-Karel Dicke proposed gluten as the contributing factor of the condition, however, these vague symptoms were the only means available for identifying the disease. Diagnosis during this time mainly relied on highly fallible markers such as steatorrhea, malnutrition, and weight loss.20 After Dicke’s gluten discovery in the 1950’s, the gluten-free diet was added as an additional diagnostic tool but this, too, proved to be insufficient measurement. Besides being relatively subjective means of diagnosis, both of these diagnostic tools only detected the classic forms of celiac disease, those in which malabsorption is apparent through overt clinical signs such as diarrhea and inability to gain weight. But not all forms of celiac disease are so obvious. The condition is additionally represented in subclinical and silent forms that display no evidence of malabsorption and
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would therefore go unnoticed by early diagnostic tools. Without accurate means of diagnosis, the reported incidences of celiac disease prior to the 1950’s therefore can only be regarded as a gross underestimations, as the vast majority of cases were surely left undiagnosed.

With the 1960’s, however, came an important revolution in terms of diagnosis. Through an examination of various intestinal specimens it was recognized that celiac disease leaves behind very unique physical and immunological changes on the mucosa of the small intestine, mainly in the form of flattened villi and intestinal lesions. Therefore in 1969, intestinal biopsy, which is now viewed as the gold standard for celiac disease diagnosis, became a necessary component in identifying the disease. In addition to the physical changes, it was also observed that celiac disease is distinguished by an increased number of plasma cells that secrete tTGA and endomysial antibodies. Both these antibodies, which target the problematic gliadin epitopes, are detectible not only in the intestine, but in the blood as well and can be recognized through serological testing. Today, intestinal biopsies and serological testing are used as the most reliable means of identifying celiac disease. Due to the newness of both of these tools as well as better understanding of the condition, many individuals speculate that any increases in the rate of celiac disease are simply a result of better diagnostic techniques and increased awareness. The application of these modern techniques to cohorts of previous generations, however, suggests otherwise.

THE CHANGING PREVALENCE

It is commonly believed that in the Western World, celiac disease affects approximately 1% of the population, a nearly five-fold increase from 30 years ago.
examine whether this increase is truly a result of greater prominence or simply greater awareness of the condition, recent studies have utilized samples of blood from previous generations to attain a more reliable estimate of past rates. One of the most revealing of these studies was conducted Rubio-Tapia et al.\textsuperscript{22} on serum collected from individuals on Warren Air Force Base (WAFB) between the years 1948 to 1954. As with modern diagnostic testing, these fifty-year-old blood samples were examined for the presence of antibodies characteristic to celiac disease, in this case tTGA and endomysial antibodies. When both of these values were shown to be positive, the presence of celiac disease was confirmed. Of the 9,133 individuals from 1948-1954, 14 met the criteria for celiac disease, a prevalence of 1 in 152 or 0.2%. To accurately compare this rate to that of modern era, the WAFB cohorts were analyzed against 1) a current population born around the same time period as the WAFB population (ages >50 between the years 1993 and 2003) and 2) a current population representing the same age of the cohort at the time of sampling (ages 19-49 between the years 2006 and 2008). Of the similar birth population, 1 in 121 or 0.8% met the criteria for celiac disease while 1 in 106 or 0.9% of the similar age population tested positive as well. As these numbers indicate, the rate of celiac disease in the US has increased four-fold, from 0.2% to 0.9%, in a little over 50 years. And because the same form of testing was conducted on all cohort groups, it is apparent that the change in prevalence is due to something more than mere diagnostic improvement.

A similar study conducted by Catassi et al.\textsuperscript{23} examined the changing disease prevalence in a single population over a shorter period of time. In Washington County, MD, blood samples collected from a cohort in 1976 and 1989 were subjected to the same serological testing used in the WAFB study. Out of a population of 3,511 subjects, seven
met the autoimmunity criteria of CD in 1976, while an additional nine tested positive in 1989. By 1989, only two of the original seven cases had been formally diagnosed, leaving 89% of the affected individuals undiagnosed. These results reveal that while diagnosis increased by only 11% in a little more than a decade, prevalence of the disease had more than doubled. This data suggests that even when taking into account the improved diagnosis of recent decades, the prevalence has still grown even more substantially. Finally, it is important to note that of the nine newly-developed cases in 1989, all were in their adult years, some even over the age of 50. Celiac disease thus changes not only over generations, but within generations.

Similar cases of celiac disease developing in the elderly have been noted in Finland. In this country, celiac disease within the adult population almost doubled from 1.05% in 1978-1980 to 1.99% in 2000-01, and within a population over 55 years of age, the prevalence rose 0.23% in only a three year period.\textsuperscript{24,25} The fact that individuals frequently test negative for celiac disease one year, and then test positive in a preceding year is indeed suggestive of a changing environmental condition responsible for the onset of the disease. And when looking at environmental change connected to celiac disease, the evolution of wheat is one of the most critical.
ENVIRONMENTAL CONNECTIONS: WHEAT DOMESTICATION AND BREEDING

Human history is replete with environmental change. It has been hypothesized that inability of humans to fully digest cereal grains is directly related to the history of modern culture far outpacing human genetics. And this is certainly true of wheat. Although it is true that wheat was not originally part of the human diet, this initial absence is only the beginning of the problem. What has further exacerbated the situation is that since its introduction, wheat has been continuously altered and adapted by human intervention; in a relatively short span of time, plant breeding has accelerated the forces of natural selection and rendered modern wheat cultivars wholly distinct from their early wild form.

Domestication is identified as the intentional and unintentional selection for certain genes. And it is this process that has carefully tailored the first wheat varieties of our ancestors into the ubiquitous staple of modern man. Although in the modern era there are at least nineteen subspecies of wild and cultivated wheat in existence, only two types dominate the agricultural arena. Today, over 95% of all wheat grown belongs to the subspecies T. aestivum (commonly known as bread wheat), while the remaining 5% belongs to T. durum (commonly known as durum or macaroni wheat).³⁶ Selective breeding practices have not only narrowed the amount of wheat species grown, they have also vastly decreased the genetic diversity among them.

ORIGIN OF WHEAT

By its very nature, wheat has great potential for genetic diversity. As a polyploid, each plant contains multiple genomes per cell, with some species exhibiting only two
genomes (diploids), while others contain up to six (hexaploids). In terms of evolution, this polyploidy was truly invaluable in enabling wheat to hybridize with two or more genomes from differentiated species, thereby massively increasing its genetic diversity.\(^1\) Such great speciation was beneficial not only in allowing the plant to adapt to a variety of environmental pressures, but also in providing farmers with a vast array of traits for breeding selection.

It is believed that the wheat genus first diverged from its progenitor species nearly 11 million years ago and that speciation within the genus began just around the start of the Neolithic Revolution 10,000 years ago.\(^1\) Much investigation has been focused on discovering the origin of wheat, but due to the massive amount of human intervention and environmental pressure since its emergence, the history is somewhat complex. Genetic analyses have determined that all wheat species can be classified as either diploid (AA), tetraploid (BBAA), or hexaploid (BBAADD), and that common to all of these species is the A genome. Based on this fact, it is believed that diploid wheat, which contains solely the A genome, was one of the first wheat species to emerge. The B genome entered species nearly half a million years later through the hybridization of uncultivated form of diploid einkorn wheat, *T. urartu*, and another wild species.\(^1,27\) In this event, tetraploid wheat, including emmer and durum wheat, came into existence.

By the time humans introduced farming, there were many types of both diploid and tetraploid wheat already in existence, all of them present within the region of the Near East known as the Fertile Crescent. Of these species, the first farmers selected diploid einkorn and tetraploid emmer wheat of Southeastern Turkey for cultivation.\(^28,29\) Because of the vast genetic diversity within the wild wheat population, these species were undoubtedly chosen
above other wild relatives due to ideal farming characteristics such as substantial storage
time, easy harvesting, and reliable yield. And in this way, even the start of agriculture
featured very early and simple form of selective plant breeding.\textsuperscript{26}

As wheat cultivation became more prominent, farmers began to place more emphasis
on selecting for traits that ensured ease of harvest. In this early selective breeding, the two
most important characteristics were the absence of spike shattering at maturity, which
prevents the loss of seeds in harvesting, and the ability of free-threshing, which enables easy
release of the grain.\textsuperscript{26} Because emmer wheat contains both of these essential characteristics,
it became the preferred variety for cultivation and kept this prominence for over 6,000
years.\textsuperscript{30}

As cultivated emmer wheat became perfected and more successful, the crop began to
expand beyond the Fertile Crescent into places such as Greece, Italy, and China.\textsuperscript{26} Through
these changing agro-ecological conditions, emmer naturally diversified. One of the most
important varieties emerging from this diversification was durum wheat, which effectively
replaced the popularity of emmer and secured its modern ranking as the most commonly
used tetraploid wheat.\textsuperscript{30} But new varieties of tetraploid wheat were not the only
developments at this time. In the Iran-Azerbaijan area, tetraploid emmer came in contact
with the wild grass \textit{Ae. tauschii} and hybridized to form the first hexaploid species.\textsuperscript{31} Such an
event was paramount in that it introduced the D genome into wheat, which has been linked
to one of the most essential qualities of the crop: superior gluten characteristics.
ROLE OF GLUTEN IN WHEAT

One of the main reasons for wheat’s popularity over other cereal grains is the unique viscoelastic properties it gives to its dough, a quality that is made possible through the gluten protein fraction of the grain. Gluten proteins exist within the endosperm of wheat and are utilized by the embryo during development. Within the grain, these proteins fractions form a tight and continuous network around the starch particles, an intricate matrix that is left behind as the starches break down during maturation. The essential quality of this matrix is that, when combined with water, the proteins come together to form an elastic, cohesive mass that has been highly valued in products such as bread, cakes, and pasta since the earliest of times. Especially in regard to leavening, gluten is essential in trapping the released carbon dioxide within its complex matrix, giving both height and stability to the final baked product. Not all wheat species, however, are equal in gluten quality. Durum wheat, which is tetraploid, produces fairly dense, heavy loaves and is far more desirable for pasta products. The unique gluten strength of hexaploid wheat, a quality wholly absent in ancestral species, is extremely desirable for bread products. And it is because of this priceless characteristic that the hybridization of bread wheat, and the introduction of the D genome, is seen as one of the greatest and most desirable accomplishments in food culture.

Over time, as wheat breeding practices became more advanced, farmers no longer had to let improvement come about through evolutionary roulette, they could directly control it. While until the 19th century wheat advancement only occurred through random crossings of various heterogeneous wheat landraces, this last century brought about the revolutionary introduction of artificial breeding practices. By and large, the main focus of these practices has been improved baking characteristics of both durum and bread wheat
cultivars. And the result has been not only a rapid increase in gluten content, but also a drastic decrease in genetic diversity of cultivars.

**SELECTIVE BREEDING IN WHEAT**

High protein content and increased gluten strength are two of the most sought-after qualities in modern wheat breeding programs, for both durum and bread wheat. It has been identified that the two components of gluten—gliadin and glutenin—both offer different essential properties to wheat dough. Gliadin units, on the one hand, tend to exist in monomers and contribute viscosity through water absorption properties and lipid interactions. These properties of gliadin are believed to be essential in creating breads of desirable size. Glutenin subunits, on the other hand, tend to interact with one another to form large molecular weight polymers that contribute both elasticity and dough strength. These polymers are classified as either low-molecular-weight (LMW) or high-molecular-weight (HMW), and provide tolerance in over-mixing as well as help maintain a product’s shape after baking. Wheat breeding programs worldwide have thus focused on optimizing the content of both gliadin and glutenin in modern cultivars for the purpose of improved baking characteristics.

Especially in major wheat producing countries such as the United States, Canada, and Italy, where the majority of wheat is exported and thus must be of the highest market value, the focus on improved baking characteristics, also known as end-use quality, is of paramount concern. Traditionally, the quantity and quality of the protein is only weakly heritable, being directly dependent on a variety of environmental and genotypic influences. The wheat industry, however, has taken direct control of such influences in order to increase
gluten concentrations to the most ideal amount. In Canada, one of the world’s top producers of durum wheat, hybridization and selective breeding first came into being in 1928, finding its true stride shortly after in the 1950’s. Since this time, the breeding program has had a particularly strong emphasis on end-use quality, requiring each new cultivar to meet certain quality standards before it is offered up for commercial production. Based on its global prominence and highly detailed breeding practices, the Canadian wheat breeding offers the perfect example of how modern cultivars are being modified genetically and qualitatively for the world market.

In order to develop a wheat cultivar of optimal gluten status, gliadin bands and glutenin subunits are among the first characteristics examined. The importance of HMW subunits in dough strength, particularly those contributed by the D genome, was determined nearly 25 years ago and has since remained a popular source of manipulation for bread wheat. Breeders have intentionally selected for these alleles and even utilized transgenesis, a form of genetic engineering, in order to increase these concentrations in wheat cultivars. Studies over the past decade have shown that such techniques have vastly improved the dough strength of modern cultivars. But HMW glutenin is not the only subject of genetic selection in wheat breeding. In terms of durum wheat, genetic analyses have focused more on gliadin, specifically the presence of gliadin γ-45, which is commonly associated with high gluten strength, and gliadin γ-42, which is associated with poorer viscoelastic properties. Screening for such genes in early generations of cultivars help breeders preserve the wheat lines that are homozygous for the desirable gliadin γ-45 and eliminate the lines with the undesired gliadin γ-42. Due to the close connection between gliadin and glutenin in inheritance, it has been postulated that gliadin γ-45 itself may not contribute the
improved gluten characteristics, but rather this end quality may be a result of specific glutenin subunits closely linked to it. Nevertheless, this genetic selection has a definite impact on the diversity of the cultivars, as it has been observed that elimination of the lines homozygous for gliadin γ-42 in the second generation reduces the wheat population size by a dramatic 44%. This process of genetic reduction continues over a period of 10 years as the wheat lines are further narrowed down based on overall volume weight, gluten viscoelasticity, and dry gluten content. Within each generation, only the most optimal 20% are selected and re-sown, creating plants that are not only more similar genetically, but also increasingly higher in gluten content.

Although genetic diversity is valued in the natural world, it is certainly not valued by the marketplace. Export markets desire wheat not only of superior gluten content and strength, but of utmost uniformity and consistency. As a result, today’s wheat cultivars have very little variety, differing by a mere 2% in their dry protein content. This is a distinct difference from wheat landraces and obsolete cultivars, which exhibit up to 88.5% heterogeneity in their protein profiles. In this way, breeding in the past fifty years has distinctly separated homogenous modern wheat from its diverse origins.

**SELECTIVE BREEDING EFFECTS**

Darwin first pointed out that plants are extremely beneficial tools for examining biological evolution in that one can perfectly observe the profound impact of plant-human interaction through a simple comparison of a plant’s domesticated and wild forms. Such is indeed applicable to wheat. Landraces in particular, which exhibit a genetic diversity uninfluenced by modern intervention, offer the ideal yardstick for measuring the influence
of plant breeding on cultivated wheat. Due to the concern of genetic erosion in wheat breeding, many studies have recently focused on comparing landraces and modern wheat cultivars in order to observe how selective breeding has changed wheat over time. And the results have indeed been telling.

The selection for improved gluten strength is a fairly recent development in breeding, particularly for durum wheat. As evidenced by the Canadian wheat breeding program, modern selective breeding was first popularized in the 1950’s and has only increased as the technology has become more developed. Comparisons in the genetic diversity of wheat over the past few decades have noticed a significant decrease in the diversity of wheat from 1950 to 1960, a loss that directly correlates with the advent of more rigorous breeding. This situation, however, has not just been noticed in genetic diversity as a whole, but in gluten content in particular.

One specific study conducted by Motzo et al. examined the genetic changes in the various eras of durum wheat grown in Italy: landraces grown up until 1950, cultivars grown between 1940 and 1970, and cultivars introduced from 1974 to 1998. All three groups were grown for two seasons and tested for protein concentration and gluten index. Protein percentage was utilized in order to determine percentage of protein to starch in the grain, while gluten index was used to measure the ability of wet gluten to remain coalesced in a sieve during centrifugation, an indication of gluten strength. When examining these two vital properties in the different eras of durum wheat breeding, it was revealed that breeding practices have indeed drastically altered modern wheat. First and foremost, although the landraces and cultivars grown between 1940 and 1970 contained around the same percentage of protein, actual gluten content was significantly lower in the landraces (9.5%
versus 10.7%). But what is perhaps most striking is in regard to the gluten index of modern cultivars. Compared to the medium 31-34% gluten index of landraces and early cultivars, modern cultivars exhibited an astonishing 86.9% gluten index, over double the amount of their earlier counterparts.\footnote{35}

Comparable results have been observed in the bread wheat of Spain. Similar to Canadian practices, Spain used mainly local landraces and cultivars in the first half of the twentieth century, which were later displaced by imported, selectively-bred varieties in the second half of the century. A study carried out by Gomez et al.\footnote{45} revealed that although protein concentrations varied little between the different eras of breeding, gluten concentrations were starkly different. Flour protein content and gluten index in particular, both key indicators of protein quality, were significantly higher in the cultivars introduced in the 1970’s compared to earlier landraces. And for the most modern of hexaploid wheat cultivars, overall protein quality was noted to exceed even highest levels observed in landraces.\footnote{46}

**CHANGING EPITOPES**

But modern breeding may not have only increased the level of gluten in modern cultivars; it may have also increased its level of toxicity. Recent studies have focused on whether the gluten composition of modern wheat varieties may be accountable for the rising number of celiac disease cases. And indeed it has been revealed that in comparison to landraces, both hexaploid and tetraploid modern wheat express a higher frequency of the most reactive celiac-inducing epitopes.
Glutenin and gliadin contribute approximately 80% of total protein content in wheat. Although both proteins are essential in baking quality, it is gliadin which has been shown to be the most problematic in regard to celiac disease. When gliadin is deamidated by TGase in the lamina propria of the small intestine, the resulting cleavage exposes T-stimulatory epitopes and triggers an immune response. These toxic epitopes are derived from three different fractions of gliadin—α-gliadin, γ-gliadin, and ω-gliadin. Although all three of these forms have the potential to initiate an immune response, it has been shown that epitopes derived from α-gliadin are the most reactive in regard to celiac disease.

It is true that all wheat contains gliadin epitopes, but certain varieties have been shown to have greater immunogenicity than others. Recent research has revealed that each wheat genome (A, B, and D) contributes very differently to epitope content. Within these differences, however, there appears to be a strong correlation between gluten strength and epitope expression. Since its first emergence, bread wheat has been highly valued for the superior baking qualities sourced from its D genome. Analysis of diploid wheat accessions, however, revealed that the D genome is responsible not only for improved gluten strength, but also for greater expression α-gliadin epitopes. In an analysis of the gliadin sequences from hexaploid wheat (AABBDD), the D genome appeared to be the preferred source of α-gliadin epitopes; all four stimulatory α-gliadin sequences—Glia-α, Glia-α2, Glia-α9, and Glia-α20—were expressed at extremely high frequencies in the D genome, while the B genome rarely contained two of the four epitopes and therefore had little T-stimulatory effect.

Hexaploid wheat is unique in that it is the only variety of wheat to contain the D genome. Looking at the history of the cereal crop’s development, hexploid varieties were a
fairly recent addition, only appearing after the agricultural revolution. But despite its late start, the value of hexaploid wheat in human history is irreplaceable. Today, bread wheat is responsible for 95% of all wheat production, and thus the seemingly toxic D genome has become pervasive in wheat-based societies. What is more, breeding practices in bread wheat have aimed to increase the qualities naturally provided by the D genome, and the result appears to be an increase in the celiac disease epitopes of modern cultivars. A study comparing two modern bread wheat cultivars, Lavett and Baldus, revealed that Lavett, with its higher baking quality of 9 (compared to the 6 of Baldus), had a higher rate D genome allele expression (62% compared to 28%) as well as almost two times the α-gliadin epitope expression (2.7 epitopes per gliadin transcript opposed to 1.6). In addition, Glia-α9, a major epitope expressed in the proteolytic-resistant 33-amino-acid sequence of α-gliadin, has been shown to be more prevalent in modern hexaploid wheats compared to landraces. In a study of 36 modern hexaploid varieties, only one modern cultivar exhibited a low expression of Glia-α9, compared to a much higher 30% of landraces.

Increasing toxicity, however, is not solely restricted to hexaploid wheat. In tetraploid wheat, which lacks the D genome of hexaploid varieties, celiac disease epitopes in the form of Glia-α9 and Glia-α20 are mainly expressed through the A genome. In an analysis of a modern durum wheat cultivar compared to more diverse tetraploid landraces, it was revealed that the frequency of T-stimulatory epitopes from the A genome was almost always higher in the modern cultivar (41%) compared to the majority of landraces (ranging between 12-58%). The breeding of modern durum and bread wheat thus appear to have contributed not only to a higher baking quality, but a higher toxicity as well.
DISCUSSION

Genetics have certainly played an important role in celiac disease development, for at the most basic level, the gluten portion of wheat is inherently incompatible with the basic genetics of human digestion. This is evident in the fact that all humans lack the necessary enzymes to break down gliadin and that nearly 40% of the population possesses alleles that still recognize gluten as a foreign entity. It appears that the sudden introduction of wheat into the human diet provided relatively little time for the species' genetics to properly adapt to the new food substance, and as a result, human digestive processes were left completely inept at adequately metabolizing wheat’s most prominent protein, gluten.

But human genetics is only the beginning of the issue. For while the possession of the HLA allele is necessary in celiac disease, the gene itself only accounts for 40-50% of disease development. Clearly celiac disease is not entirely determined by genetic makeup; while cereal grains have been a part of the human diet for nearly 10,000 years, celiac disease has only recently become an increasing problem. Additionally, reports have shown that although celiac disease may have existed as far back as the first century AD, the doubling and quadrupling in its prevalence has only occurred within the past fifty years. This changing disease trend suggests that there is a recent factor that has contributed to the exacerbation of this long-standing issue—a modern development that has added to initial dietary issues started by the agricultural revolution 10,000 years ago. And evidence seems to indicate that this contributing factor is the prominence of gluten within the diet.

At the very start of the agricultural revolution, the varieties of wheat chosen for cultivation were fairly diverse. Unlike modern times, initial farming practices utilized landraces, which are the natural result of biotic and abiotic stresses of the environment. Such
landraces exhibited great genetic diversity, differing in characteristics such as grain size, protein content, and gluten strength. Farmers would plant large collections of different wheat genotypes, letting natural selective processes choose the varieties best adapted to the environment to survive and reproduce. In the first half of the twentieth century, these landraces remained the primary form of wheat used in farming. And up until this time, celiac disease, although existent, was a fairly rare disorder. Between the 1920’s and 1950’s, however, came a prominent shift in the way in which wheat was bred. Modern breeding practices moved away from the natural selection approach to that of pure human intervention. And the product of this change was a monoculture of “pure wheat genotypes” that were heavily selected for uniformity and superior gluten characteristics. As a result, after the 1950’s, wheat became not just more genetically homogenous, but also significantly higher in gluten content.

Sweden is perhaps one of the best examples of how increasing the gluten content can adversely affect disease prevalence. From 1984 to 1996, Sweden experienced an epidemic in celiac disease directly related to the changing infant feeding practices. In 1984, recommendations suggested that high amounts of gluten should be introduced fairly abruptly at 6 months of age. At the same time, the gluten content of commercially-available milk cereals and porridges was increased two-fold. As a result of both of these changes, the prevalence of celiac disease among Swedish two-year-olds quadrupled in only a few years. Later on, children born during this epidemic exhibited a disease prevalence of 3% by age 12, a rate far higher than any other country has ever experienced. In 1996, feeding practices were changed again in order to address this problematic epidemic. Gluten was suggested to be introduced more gradually and as a result, disease prevalence declined to its
pre-epidemic level. Studies since this period have revealed that introducing large amounts of gluten compared to smaller amounts is associated with a 50% increased disease risk, thereby supporting the connection between dietary gluten and disease prevalence.

Further evidence to support this correlation comes from a comparison of Sweden to Denmark in 1995. Due to the close geographic proximity of the two countries and their nearly identical standards of living as well as geographic and cultural backgrounds, it is interesting to note that the incidence of celiac disease is over 30 times higher in Sweden. In Sweden, the average child is diagnosed with celiac disease at 1.5 years of age, while in Denmark it is much later at 5.5 years. But where there are such great cultural and genetic similarities between the two populations, one substantial difference exists, and that is in regard to diet. At 8 months of age, it has been estimated that the Swedish diet contain 40 times more gliadin than the Danish diet. And while Sweden is based heavily on high-gluten wheat as is main carbohydrate source, the Danish diet is founded on the lower gluten-containing rye flour. The country of Sweden therefore represents a perfect model for examining how the increased presence of gluten in the diet is directly correlated with celiac disease risk.

As wheat in the Western World has become more heavily selected for superior baking quality, the prominence of gluten in the diet has consequently increased. And just as in Sweden, celiac disease rates have been affected as a result. Prior to the use of heavily selective breeding in the 1950’s, celiac disease only represented an estimated 0.2% of the US population. But as the breeding practices became more developed, a notable change could be seen not just in the wheat, but in the prevalence of the disease as well. In major wheat-exporting countries such as Canada, the gluten content of wheat crops more than
doubled between 1950 and 1998.\textsuperscript{35} During this same period, the prevalence of celiac disease in the US quadrupled, while in the UK it jumped from a rare 1:8000 individuals to a much more common 1:100.\textsuperscript{52} Similarly, in the elderly Finnish population, many individuals born between the years 1926 and 1950 had no incidence of celiac disease during their youth, but only developed the disease fifty years later, after wheat had been altered for superior gluten content.\textsuperscript{25} As humans have continued to introduce a cultivar higher and higher in gluten content into their diet, the prominence of celiac disease has skyrocketed.

This increasing disease rate, however, may not be solely due to greater gluten content, but also in increasing gluten toxicity of wheat. Analyses of modern cultivars have revealed that newly selected wheat varieties exhibit a much higher frequency of the most reactive celiac disease epitopes compared to landraces. In Canadian wheat breeding, improved end-use quality of durum wheat is defined by higher amounts of $\gamma$-gliadin, which has been shown to cause reactions in many celiac patients. And in relation to bread wheat breeding, HMW and LMW glutenin subunits, the main foci of improved bread quality, are also suspected to activate T-cells in some celiac patients.\textsuperscript{53} Superior wheat characteristics thus appear to be intimately tied to higher toxicity for celiac disease patients. Modern breeding practices over the past fifty years, it appears, may be playing a major role in the increasing prevalence of celiac disease by providing both historically high amounts of as well as greater toxicity in gluten in the diet.
CONCLUSION

Much like the agricultural revolution of 10,000 years ago, modern wheat breeding has presented both advantages and disadvantages to human society. Just as the agricultural revolution provided great technological and social developments to human civilization 10,000 years ago, so too did the introduction of selective breeding in the 1950’s provide humanity with a more desirable, higher quality wheat product—but at an apparent consequence to human health.

In both cases, it appears that human health has been compromised by human intervention. First and foremost, the introduction of farming 10,000 years ago represented the beginning of human control over the species’ food supply. Humans no longer had to be dependent on the unpredictable sources of hunting and gathering, but could instead grow their own. With this more controlled sourcing, wheat became the primary caloric contributor in the human diet, effectively displacing the much more fiber- and nutrient-rich fruits and vegetables of Paleolithic times with a single starchy crop. The consequence of this change, however, was that the human diet lost an important source of essential nutrients, leading to an increase in anemia, osteomalacia, and dental caries as well as a decrease in stature of post-agricultural humans.\textsuperscript{7,8} As the centuries have progressed, this direct control in food supply has only continued as wheat breeding practices have become more rigorous. The modern breeding techniques initiated in the 1950’s are a perfect example of this, as they use human intervention as a necessary tool for creating the perfect wheat cultivar. The unfortunate consequence of such selective practices, however, has been an increase the prominence and toxicity of wheat’s most problematic protein: gluten. And as a result, celiac disease prevalence appears to have increased.
There may, however, be a way to use human intervention to reverse such problematic trends. With better understanding of the link between wheat breeding and celiac disease prevalence, it may be possible to use modern techniques to create a less reactive wheat cultivar. Science has identified some of the most sensitive celiac disease epitopes and even located where they exist in the wheat genome. By focusing efforts on understanding how these epitopes are inherited, researchers have noted that it may be possible to create a high-quality, low-toxicity wheat in the future. In this way, human intervention, the very source of the problem, may actually offer the future solution to celiac disease prevalence.
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