Effect of Gluten Supplementation in Healthy Siblings of Children With Celiac Disease

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The clinical, functional, and histopathological effects of 18 g additional gluten intake daily for 4 wk was studied in 13 healthy siblings of Spanish children with celiac disease, including two pairs of discordant monozygotic twins. Five of the 13 children were HLA-DR identical to the celiac sibling, 5 shared only one HLA-DR antigen with the celiac sibling, and 3 had completely different HLA-DR antigens than their respective celiac brothers or sisters. Clinical evaluation and functional tests (routine blood, xylose absorption, and fecal fat excretion studies) were performed before, during, and after gluten challenge. A jejunal biopsy specimen was taken at the end of the 4-wk period of gluten supplementation. No clinical abnormalities were found during the period of the study and there was no significant decrease of xylose absorption. Fecal fat excretion studies gave normal results, both before and after gluten challenge. The high gluten diet did not lead to histopathologic abnormalities in any of the jejunal biopsy specimens, which showed a normal range of crypt to villus ratio and surface-cell height. The present results do not support the view that excessive gluten intake is toxic for individuals who are genetically predisposed but do not have overt celiac disease. The findings also suggest that other factors besides HLA-DR antigens and gluten intake are important for expression of the disease.

Genetic and environmental factors are known to play a role in the pathogenesis of celiac disease. The most important of these factors identified so far are the presence of gluten in the diet and the HLA-DR antigens, but it is not known whether, and if so how, such factors interact to produce the disease state. Studies of families with celiac disease have revealed a number of healthy siblings sharing identical HLA-DR haplotypes with their celiac brothers and sisters (1). Some authors have suggested that the effect of gluten intake in susceptible individuals without overt small bowel disease, including first-degree relatives of celiac patients and patients with an immune deficiency syndrome, may be dose-dependent (2). However, both a long-term study carried out by Levine et al. (3), who gave large amounts of extra gluten daily to healthy individuals, and a more recent study on the effect of direct gluten instillation into the small intestine of nonceliac individuals during a 24-h period by Bramble et al. (4) found no lesions in the intestinal mucosa after the gluten challenge.

The present study was carried out to ascertain whether the consumption of supplementary quantities of gluten (18 g extra per day) during a period of 4 wk would alter gastrointestinal absorption or have a toxic effect on the mucosa in a group of celiac siblings thought to be at special risk of developing celiac disease.

Subjects and Methods

Thirteen healthy siblings (5 girls, 8 boys; mean age 10.5 yr) of 12 Spanish children with celiac disease were studied. Two of the subjects were the healthy members of two sets of monozygotic twins discordant for celiac dis-
HLA-DR haplotypes were identical to those of their celiac sibling; 5 others had only one such identical haplotype, and 3 children had HLA-DR haplotypes differing completely from those of their celiac brothers or sisters.

The jejunal biopsy specimens taken from 3 healthy siblings before the study showed no histopathological abnormalities. The results of the morphometric evaluation of villus length, crypt depth, crypt to villus ratio, and surface-cell height in 11 of the jejunal biopsy specimens taken after the 4-wk period of extra gluten intake are shown in Table 1. No significant differences were found among the various HLA-DR categories of the healthy siblings, and the morphometric values are within the normal range (villus height, 313–512 μm; crypt height, 114–231 μm; enterocytes, 30–40 μm) published by Lee and Toner (8).

Table 2 shows the results of the comparison of the

Results

The results of HLA-DR typing of the 12 families are shown in Figure 1. In 5 of the healthy children, including the 2 monozygotic twins, both HLA-DR haplotypes were identical to those of their celiac siblings; 5 others had only one such identical HLA-DR, and 3 children had HLA-DR haplotypes differing completely from those of their celiac brothers or sisters.

No clinical alterations were found in any of the children before, during, or after the period of extra gluten intake. The mean values (± standard deviation) of the xylose absorption and fecal fat excretion studies in the group of healthy children with HLA-DR identical to that of their celiac siblings both before and after a 4-wk period of 18 g of extra gluten per day were 34.2 ± 5.1 to 32.2 ± 4.4 mg% and 2.2 ± 0.7 to 2.6 ± 0.6 g/24 h, respectively. The results of similar studies performed in the groups of healthy children with one HLA-DR identical and no HLA-DR identical to their celiac siblings were 29.6 ± 2.1 to 30.4 ± 2.1 mg% and 2.9 ± 0.7 to 2.7 ± 0.6 g/24 h for the former and 34.0 ± 5.0 to 32.3 ± 5.7 mg% and 2.0 ± 0.6 to 2.2 ± 0.6 g/24 h for the latter. No significant differences were found among the three different groups according to Student’s t-test.
morphologic features studied in the healthy siblings with those of a control group of 10 children without small intestinal alterations who were on a normal diet. With the exception of the mean crypt to villus ratio, which was higher for the healthy siblings than for the control group, no significant differences were found between the two groups.

Discussion

It is well established that gluten has a deleterious effect on the intestinal mucosa of celiac patients, even in the absence of symptoms (9). In patients with dermatitis herpetiformis it has been shown that the small intestinal lesions produced by gluten are dose-dependent (10). However, the question of gluten toxicity in nonceliac individuals is still controversial. In 1966, Levine et al. (3) found no morphologic abnormalities in the jejunal biopsy specimens of a group of 26 nonceliac adults after at least 8 wk of extra gluten intake (100–150 g/day), whereas Doherty and Barry (2) more recently reported that 40 g of extra gluten daily for 6 wk produced immunohistologic alterations in the small intestinal mucosa of relatives of celiac patients and patients with altered immunity. The latter study suggested that gluten alone is toxic for susceptible individuals without overt celiac disease. As identical twins and siblings of celiac patients, especially those with identical HLA-DR antigens (1), have a higher risk of developing celiac disease, it was of interest to find out whether extra gluten intake would produce clinical, functional, or histologic abnormalities in these children. In our studies, 18 g of extra gluten daily for 4 wk failed to produce any alterations in a group of 13 healthy siblings of celiac children, including two pairs of discordant monozygotic twins (5). It should be noted that even where a significant difference was found between the crypt to villus ratio of the challenged healthy siblings and the control group, both ratios are in the normal range reported in healthy populations of temperate climates (Table 2) (8). The absence of alterations was independent of the HLA-DR status of the healthy siblings, whether they were HLA-DR identical to the celiac children or had HLA-DR3 and HLA-DR7, the HLA-DR haplotypes usually associated with celiac disease.

There are several possible explanations for the present findings. The first is that gluten toxicity is not dose-dependent, even for individuals with a genetic predisposition for celiac disease. In the present study only the HLA-DR antigens were taken into account, but other genetic markers, such as the Cm phenotypes of the heavy chain of immunoglobulins, are known to play a role in the immune response to gluten (11) and might have exerted an influence on the effect of the extra gluten consumed by the children. However, the absence of alterations in the discordant homozygous twins of two celiac patients after the gluten challenge seems to argue against this possibility. It is also possible that larger quantities of gluten supplementation are necessary to produce

<table>
<thead>
<tr>
<th>HLA-DR antigens in relation to the celiac children</th>
<th>Villus length (μm)</th>
<th>Crypt depth (μm)</th>
<th>Surface-cell height (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both identical</td>
<td>415.5</td>
<td>135.7</td>
<td>34.2</td>
</tr>
<tr>
<td>SD</td>
<td>±57.3</td>
<td>±21.2</td>
<td>±5.2</td>
</tr>
<tr>
<td>One identical</td>
<td>356.3</td>
<td>109.4</td>
<td>37.2</td>
</tr>
<tr>
<td>SD</td>
<td>±47.7</td>
<td>±20.1</td>
<td>±5.1</td>
</tr>
<tr>
<td>Neither identical</td>
<td>452.7</td>
<td>106.0</td>
<td>32.6</td>
</tr>
<tr>
<td>SD</td>
<td>±36.3</td>
<td>±14.3</td>
<td>±3.1</td>
</tr>
<tr>
<td>X</td>
<td>427.0</td>
<td>116.1</td>
<td>34.8</td>
</tr>
<tr>
<td>SD</td>
<td>±52.4</td>
<td>±21.2</td>
<td>±4.7</td>
</tr>
</tbody>
</table>

CV, crypt/villus ratio; X, mean value; SD, standard deviation. *Mean of five measurements or counts. Student's t-test comparing all three groups showed no significant difference.

Table 2. Morphometric Features of the Jejunal Biopsy Specimens of the Healthy Siblings of Celiac Patients, Taken After 4 Weeks With an Extra 18 Grams of Gluten Daily, and of a Control Group on a Normal Diet

<table>
<thead>
<tr>
<th></th>
<th>Villus length (μm)*</th>
<th>Crypt depth (μm)*</th>
<th>Mean CV*</th>
<th>Surface-cell height (μm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siblings</td>
<td>382.4</td>
<td>119.2</td>
<td>0.32</td>
<td>37.4</td>
</tr>
<tr>
<td>SD</td>
<td>±52.9</td>
<td>±21.2</td>
<td>±0.44</td>
<td>±4.7</td>
</tr>
<tr>
<td>Controls</td>
<td>385.0</td>
<td>105.7</td>
<td>0.28</td>
<td>37.3</td>
</tr>
<tr>
<td>SD</td>
<td>±55.0</td>
<td>±18.2</td>
<td>±0.05</td>
<td>NS</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

CV, crypt/villus ratio; NS, not significant; p, probability. Student's t-test; SD, standard deviation. *Mean of five measurements or counts.
intestinal alterations in some of the predisposed individuals without overt celiac disease. Nevertheless, the same quantity and sort of gluten intake as the one used in the present study was enough to produce histopathological alterations in 17 children who had to be challenged for diagnostic purposes.

Another possibility is that the age at which the extra gluten challenge occurs is of importance; i.e., there could be an age effect with respect to the small intestinal mucosa of predisposed individuals. Therefore, the discrepancy between our findings and those of Doherty and Barry (2) may be due to the fact that in the latter study adults were challenged, and some of them had immunologic disorders possibly acquired after childhood.

Another explanation could be that in combination with gluten, environmental factors such as intestinal viral infections, as suggested by the studies done by Kagnoff et al. (12), play a role in the expression of celiac disease. If this should prove to be the case, the age of a genetically predisposed individual and the number of contacts with intestinal viral pathogens would be of importance in the assessment of the intestinal effect of the gluten challenge.

Our present results extend the observations reported by Leigh and Marsh (13) and Bramble et al. (4), who did not find any alterations in the jejunal mucosa of nonceliac individuals after direct instillation of gluten fraction III, but found intestinal abnormalities when they tested celiac patients in this way in short-term feeding studies.

The present results do not support the view that gluten alone is toxic for individuals genetically predisposed to develop celiac disease, such as identical HLA-DR siblings or monozygotic twins of celiac patients. Other genetic or environmental factors, or both, apart from gluten intake and HLA-DR antigens probably play a role as well in the etiopathogenesis of the characteristic small intestinal lesions and in the expression of celiac disease.

References