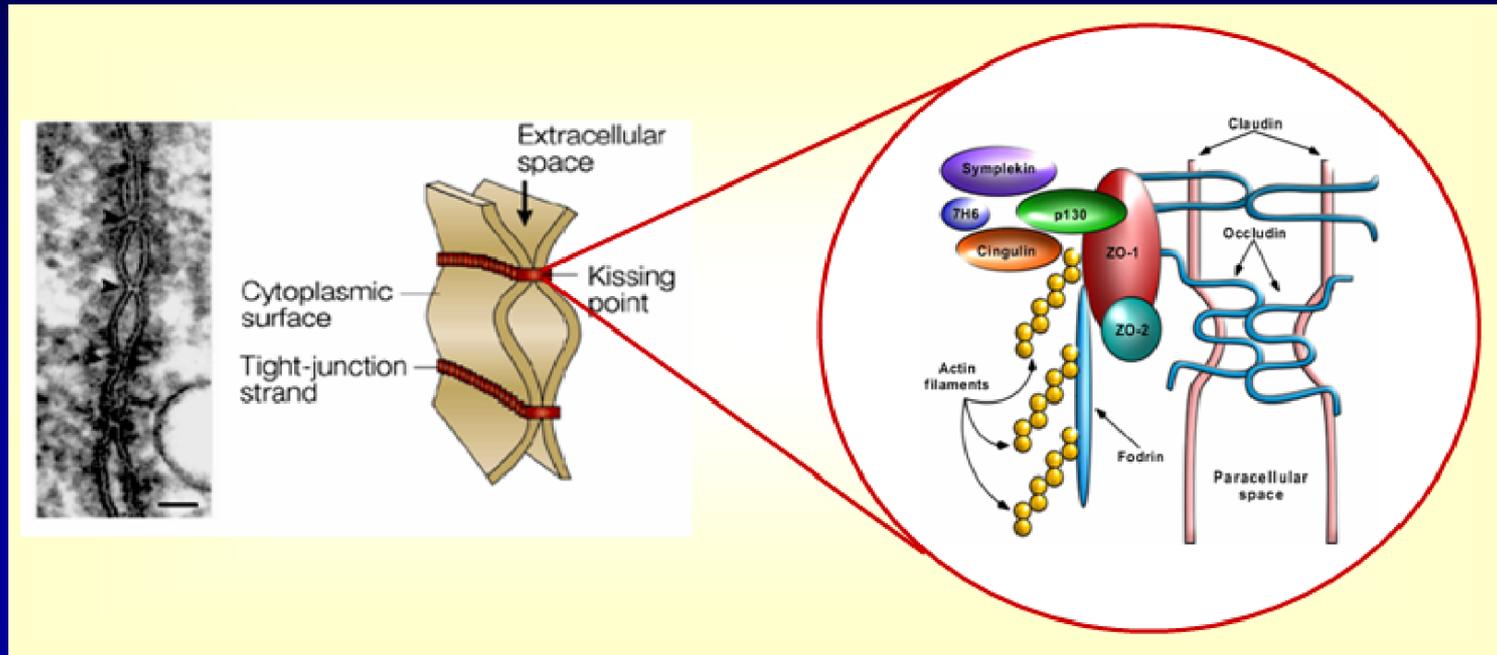


# Background

**Gliadin** is a protein responsible for the intestinal damage of **Celiac Disease (CD)**. Many studies demonstrated that CD is associated with an altered intestinal permeability. **Zonulin**, a novel protein that modulates the intestinal permeability by altering **tight junctions (tj)** competency, is up-regulated in CD. In recent years much has been discovered about the genetic and immunologic aspects of the disease, however the mechanism(s) through which gliadin crosses the intestinal barrier to reach the intestinal serosa are still poorly understood.

# Background



In intestinal enterocytes the paracellular movement of ions and macromolecules is primarily regulated by tight junctions. **Tight junctions** are dynamic intercellular structures and only recently, some of their molecular components have been identified. Proteins that constitute the tight junction complex include transmembrane proteins such as **occludin**, **claudin(s)** and junction adhesion molecules. These proteins are functionally linked to the actin cytoskeleton via scaffold proteins such as **zonula occludens 1 (ZO-1)**.

# Aims:

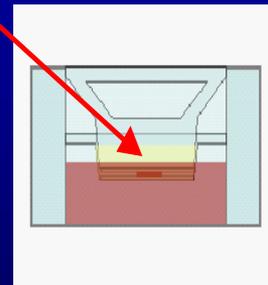
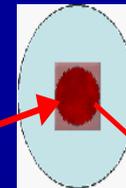
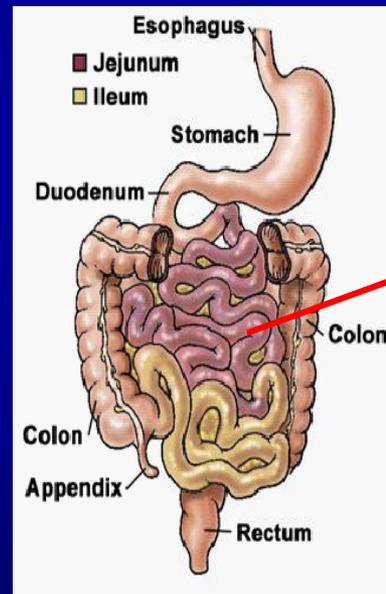
To investigate the role of gliadin on **Zonulin** release and, consequently, tight junction (tj) competency;

To establish whether gliadin affects the expression of the junctional proteins **occludin** and **ZO-1**.

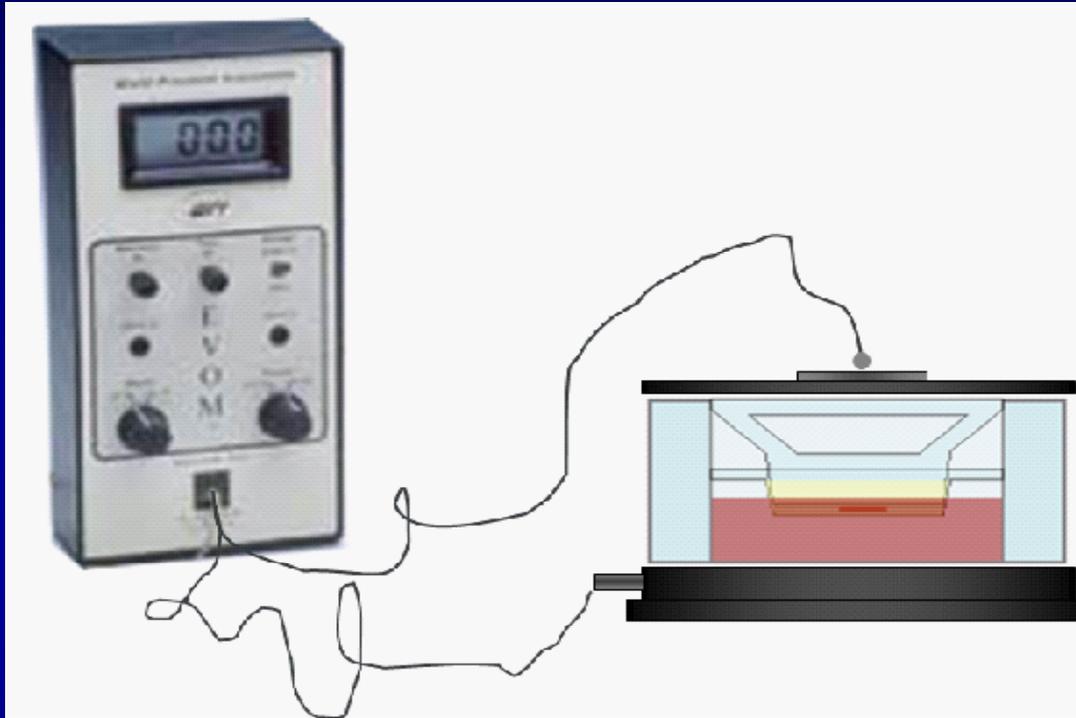
# Methods

Sixteen CD patients, 10 on gluten free diet and normal intestinal mucosa (mean age 24), five untreated subjects with partial villous atrophy (PVA), 1 with total villous atrophy (TVA), and 4 healthy controls (mean age 23) were enrolled in this study

Intestinal biopsies were mounted in the microsnapwell system as previously described by our group (1). Briefly, intestinal biopsies were sandwiched between two plexiglass dishes with an opening hole of 7 mm<sup>2</sup> area. The tissue was then mounted on the snapwell device in order to separate two compartments: the basolateral compartment and the apical compartment. PT-Gliadin was digested with pepsin and trypsin and then added to the apical compartment of the snapwell at increasing incubation times. The basolateral compartment was filled with DMEM medium used for organ cultures. Duplicate tissues were treated with bovine serum albumin (BSA) as a negative control.

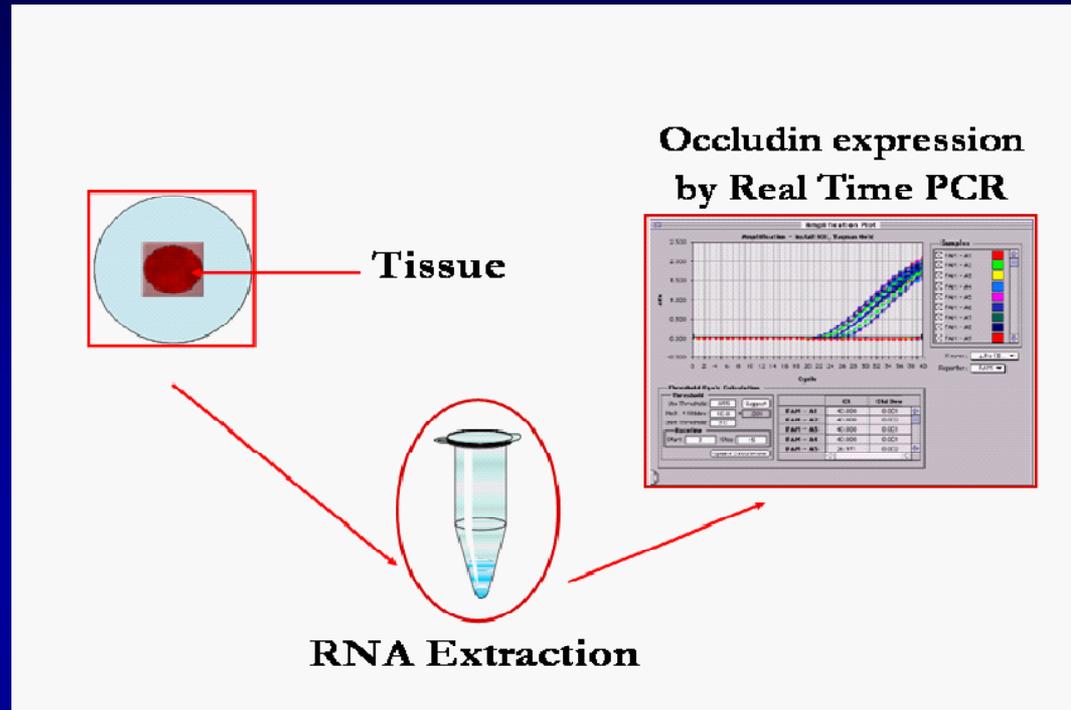


# Methods



For each incubation time, **Transepithelial Electrical Resistance (TEER)** was monitored by using the transwell device (see figure) and the secretion of Zonulin in the supernatant was detected by ELISA

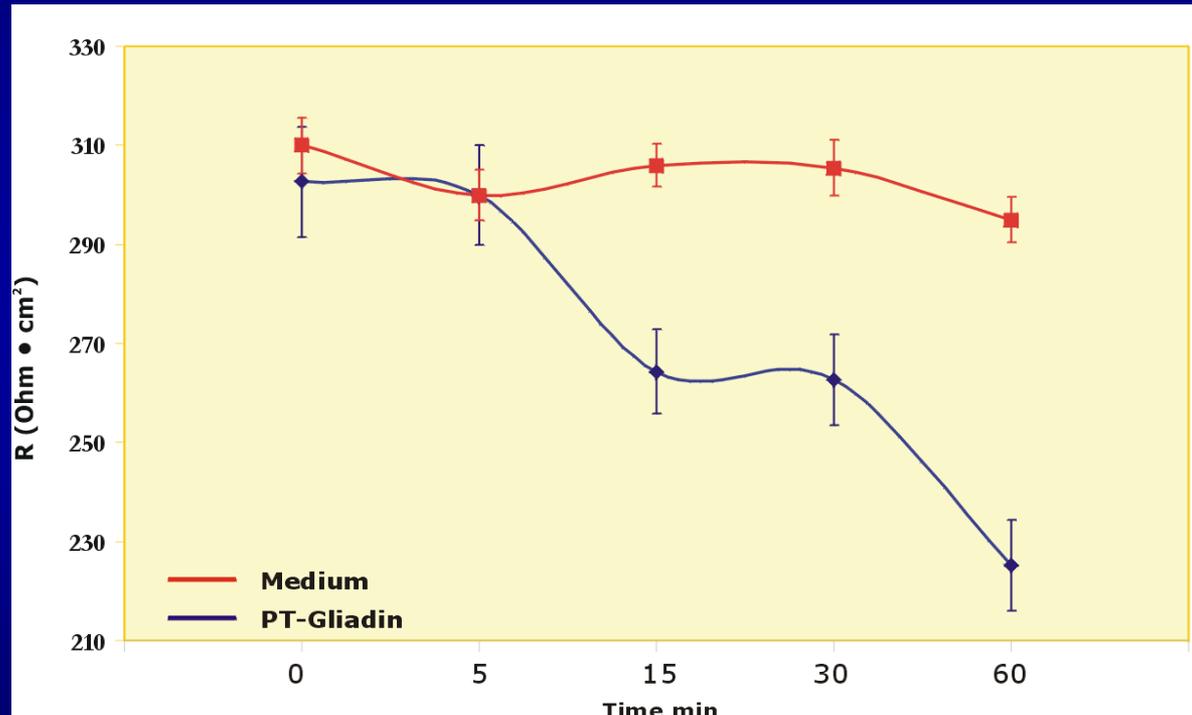
# Methods



At increasing time intervals, tissues were removed from the **microsnapwells**, the total RNA extracted and the reverse transcription of RNA to cDNA was performed. **Occludin** and **ZO-1** RNA levels were evaluated by PCR Real Time using the TaqMan probe technique (see figure). Both occludin and ZO-1 expression were also evaluated on intestinal biopsies obtained from CD patients (both treated and untreated) and controls.

# Results I

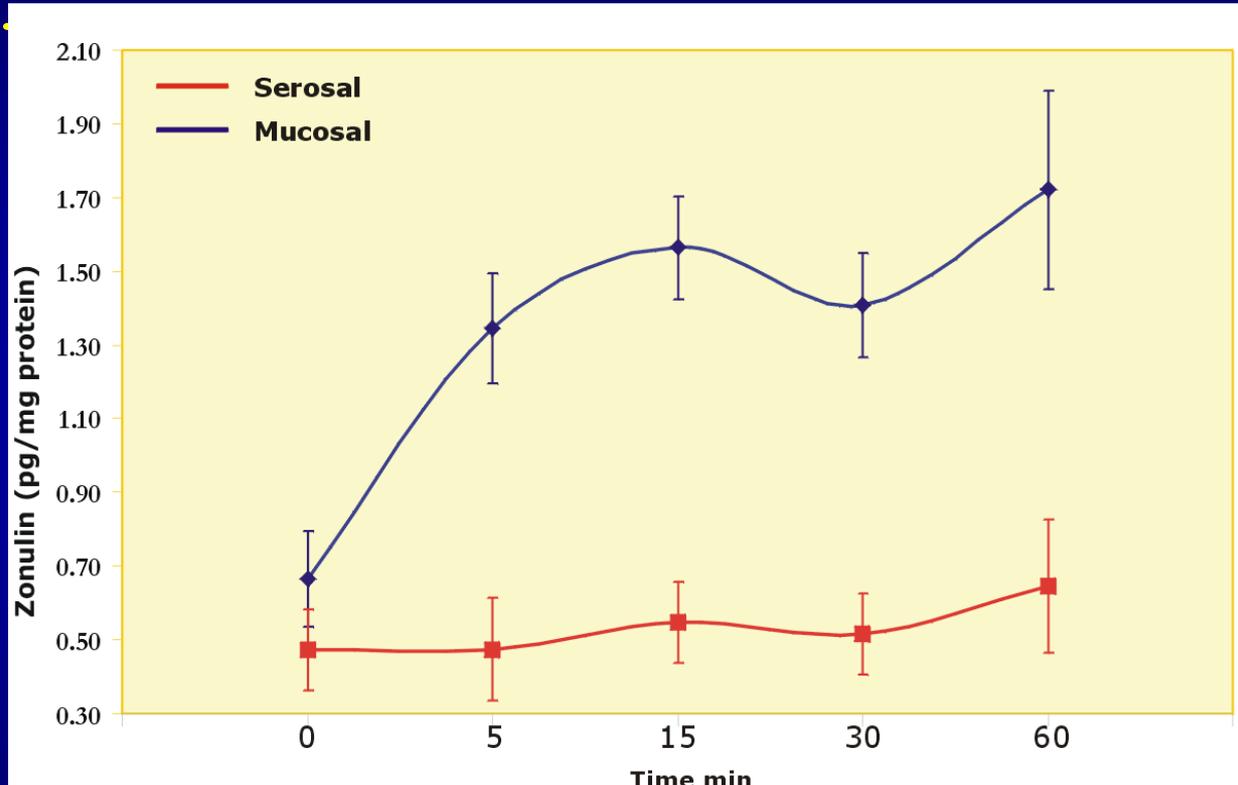
Transepithelial Electrical Resistance (TEER) in intestinal mucosa from treated CD patients mounted in microsnapwells and exposed to either media control (red line) or PT-gliadin (blue line).



PT-gliadin induced a significant TEER decrement that started as soon as 15 min post-exposure. No significant change were detected in media control-exposed tissues.

# Results II

Zonulin release in the mucosal (blue line) or serosal side (red line) from treated CD patients mounted in microsnapwells and exposed to PT-gliadin.



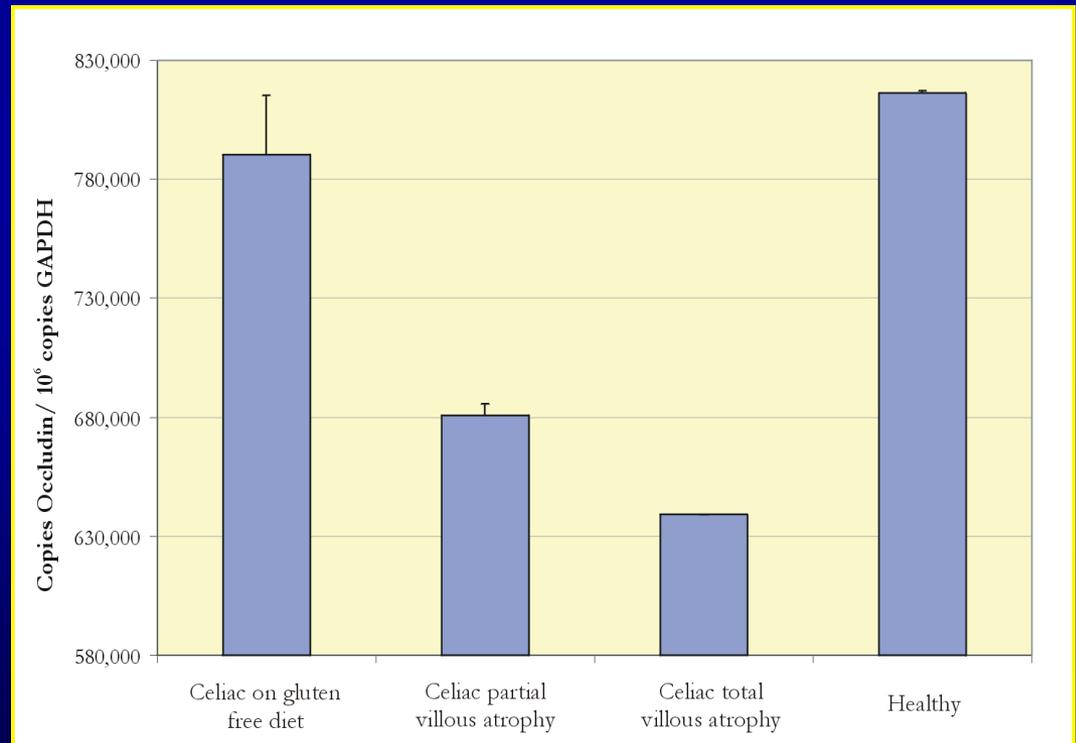
PT-gliadin induced zonulin release in the mucosal but not serosal side as soon as 5 min post-exposure. Zonulin release temporally preceded TEER decrement.

# Results III

Occludin RNA levels in CD patients (treated and untreated) and controls

The occludin RNA basal levels were evaluated in CD patients on gluten free diet with normal intestinal mucosa (10), untreated patients with partial atrophy villous (5), and one subject with total atrophy villous. Four subjects that underwent to upper endoscopy because of dyspeptic symptoms and normal intestinal mucosa were used as healthy controls.

The results are reported as number of occludin RNA copies normalized by the housekeeping gene GAPDH. Occludin RNA basal levels in healthy controls were comparable to that detected in CD patients on a gluten-free diet. The number of copies of occludin RNA detected in CD patients or total villous atrophy with either partial or total atrophy was significantly lower than that detected in treated patients and paralleled with the severity of intestinal damage.

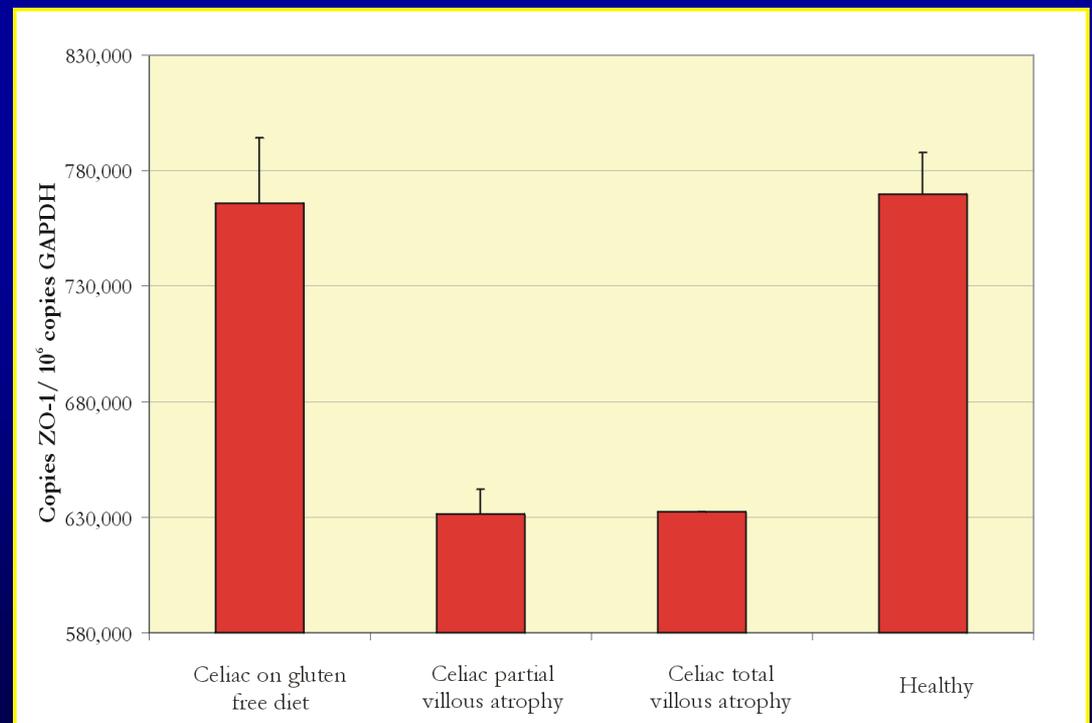


# Results IV

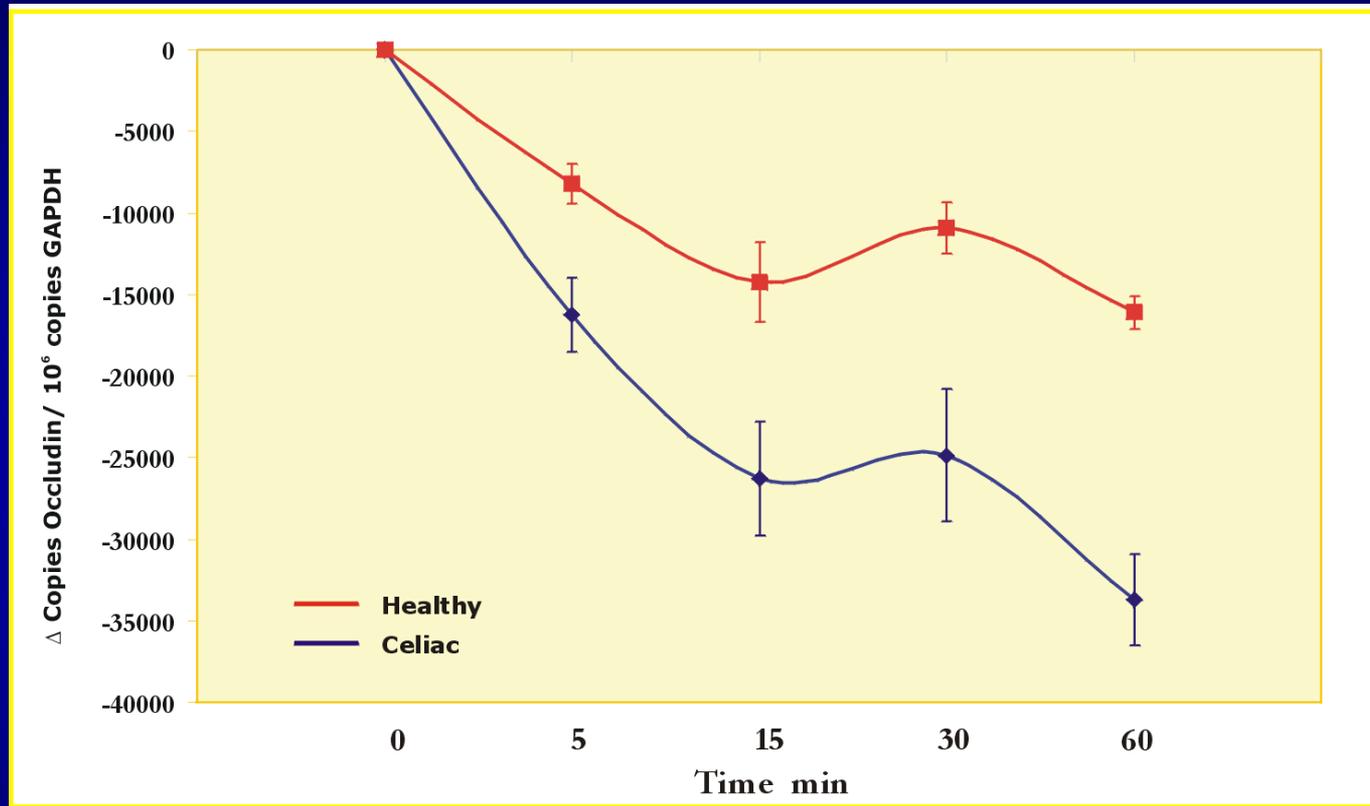
## ZO-1 RNA levels in CD patients (treated and untreated) and controls

The ZO-1 RNA basal levels were evaluated in CD patients on gluten free diet with normal intestinal mucosa (10), untreated patients with partial atrophy villous (5), and one subject with total atrophy villous. Four subjects that underwent to an upper endoscopy because of dyspeptic symptoms and normal intestinal mucosa were used as healthy controls.

The results are reported as number of ZO-1 RNA copies normalized by the housekeeping gene GAPDH. ZO-1 RNA basal levels in healthy controls were comparable to that detected in CD patients on a gluten-free diet. The number of copies of ZO-1 RNA detected in CD patients with either partial or total villous atrophy was significantly lower than that detected in treated patients..



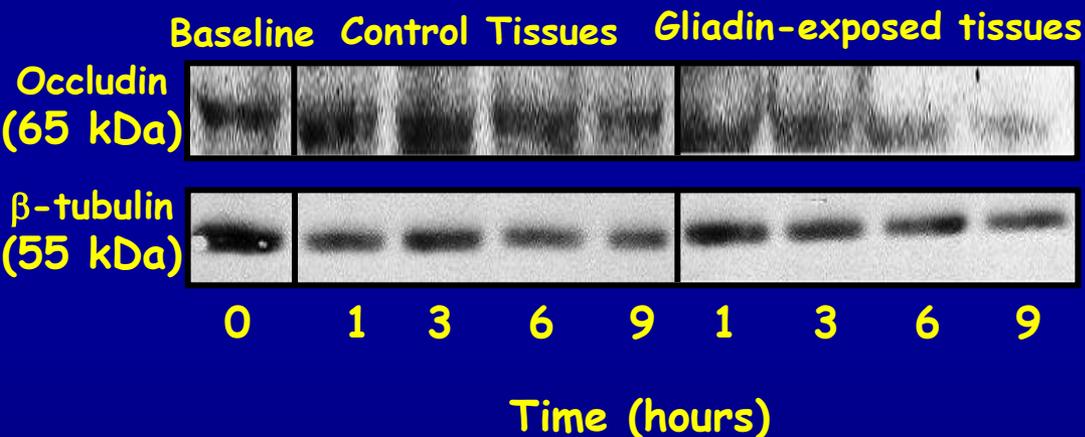
# Effects of gliadin on occludin expression from intestinal biopsies mounted in the microsnapwell system.



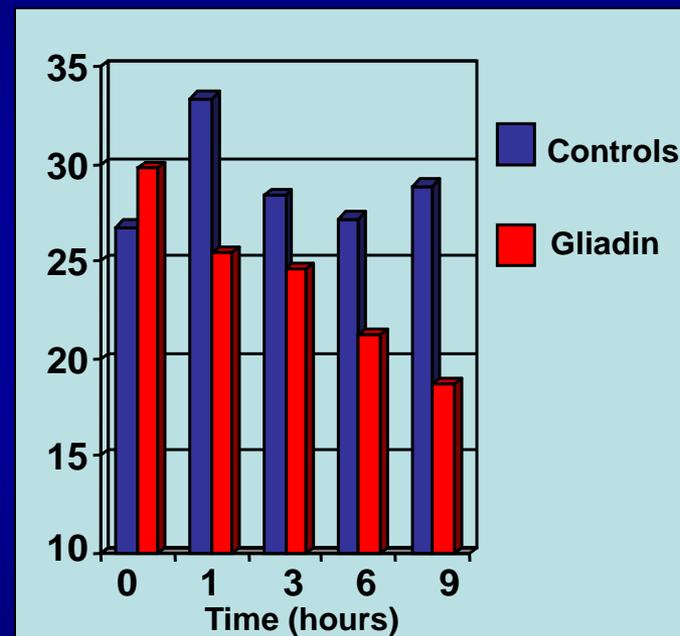
PT-gliadin induced a decrease of occludin expression in both CD patients (blue line) and controls (red line). The decrement in CD subjects appeared significantly higher than in normal subjects.

# Effects of gliadin on occludin protein expression in intestinal biopsies from CD patients mounted in the microsnapwell system.

## Western immunoblotting

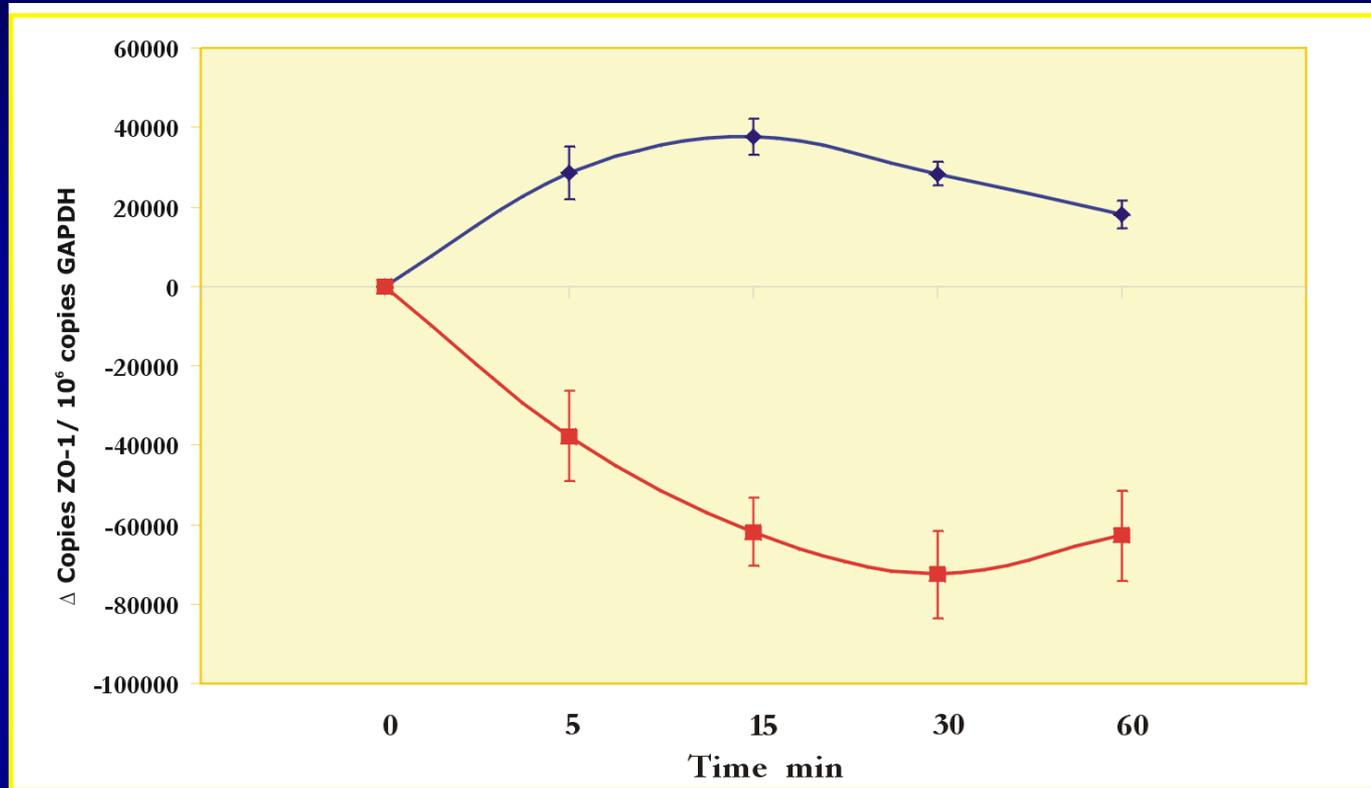


## Densitometry



PT-gliadin induced a decrease in occludin protein expression in intestinal tissues from CD patients on GFD starting 6h post-exposure. No changes were detected in tissues exposed to buffer control.  $\beta$ -tubulin was used as house keeping protein to ensure equal protein loading.

# Effects of gliadin on ZO-1 expression from intestinal biopsies mounted in the microsnapwell system.



PT-gliadin induced an increase of **ZO-1 expression** in CD patients on GFD and normal intestinal mucosa (blue line), while a significant decrease of ZO-1 expression was observed in healthy controls (red line). These changes were detectable as early as 5 min post-exposure

# Conclusions

The active phase of CD is characterized by down-regulation of expression of both ZO-1 and occludin junctional proteins. This down-regulation is reverted by gluten-free diet;

Acute gliadin exposure induces polar (i.e. luminal) zonulin release from intestinal biopsies of CD patients mounted in the microsnapwell system;

This release was temporally coincident with changes in occludin and ZO-1 junctional proteins expression and TEER decrement;

Compared to healthy controls, CD intestines exposed to gliadin showed an immediate down-regulation of occludin mRNA, followed after 6 hr by a down-regulation of the protein expression.