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## A Peptide from Kiwifruit Exerts Anti-Inflammatory Effects in Celiac Disease Mucosa

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### ABSTRACT

**Objective:** Celiac disease is an immune-mediated disease of the intestine triggered by gluten. Gluten elicits, in genetically susceptible individuals, cytokine responses that are then transmitted to the immunocompetent cells. Vegetables and fruit have anti-inflammatory and antioxidant properties with a protective effect on intestinal epithelium. Kiwifruit is known to have beneficial effects on the intestinal tissues, and it is the only plant food containing the peptide kissper, with anti-inflammatory properties. The aim of this study was the evaluation of the kissper effect on the gluten-induced inflammation in celiac disease.

**Methods:** We used an in vitro model of intestinal culture explant from celiac disease patients and non-celiac disease patients, cultured for 24 hours with the toxic gliadin peptide P31-43 and kissper preincubation.

**Results:** Our data showed HLA-DR and TG2 reduction in the celiac disease mucosa pretreated with kissper, as well as a reduction of COX-2 in two patients. No differences we observed for the TGF- $\beta$ 1 and IL-15 levels in supernatants upon kissper pretreatment.

**Conclusions:** The preliminary results suggest that kissper has a potential anti-inflammatory role in celiac disease.

### ARTICLE HISTORY

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### KEYWORDS

Celiac disease; intestinal culture explant; kissper; inflammatory mediators; diet

### Introduction

Celiac disease (CeD) is a food-intestinal disease frequently observed in Western countries (1%). The disease occurs in genetically predisposed people positive for either human leukocyte antigen DQ2 or human leukocyte antigen DQ (1). The disease is related to the intake of gluten, contained in wheat, rye, and barley. The gluten contains the soluble gliadins and the insoluble glutenins. As reported, “The extracellular matrix enzyme tissue transglutaminase 2 (TG2) selectively modifies gliadin peptides, through deamidation inducing the adaptative immune response. These modified peptides have an elevated affinity for human leukocyte antigen (HLA) DQ2 and DQ8 molecules that cause the T cell activation and finally the mucosa damage (2, 3). In our in vitro study, however, we use the P31-43 peptide (from alpha-gliadin) which induces the inflammation through the innate immune response pathway in mucosal explants from celiac patients, as already shown in several previous studies” (4). During the inflammatory process, the intestinal surface expresses proteins such as human leukocyte antigen-antigen D related (HLA-DR) and cyclooxygenase 2 (COX-2) and the interleukin-15 (IL-15) production increases, delivering signals that drive chronic inflammation (5). In fact, IL-15 is

hyperexpressed in intestinal epithelium and lamina propria of untreated celiac patients compared with healthy controls and patients who follow a gluten-free diet (6).

Transforming growth factor beta (TGF- $\beta$ 1) signaling also increases, counteracting the proinflammatory signals. TGF- $\beta$ 1 seems to have an important role in the control of mucosal inflammation in CeD, as well as in inducing oral tolerance and mucosal immunoglobulin (Ig) A responses (7, 8).

Some food components, including plant polyphenols, vitamins, and fatty acids, may have a role in the nutritional therapy of gut inflammation (9, 10). Among plant foods, kiwifruit is one of the best sources of antioxidants, such as vitamin C, polyphenols, and superoxide dismutase (SOD)-like activity component (11). In fact, it seems that the consumption of a kiwifruit (100–150 g) is sufficient to provide the entire daily dosage of antioxidants recommended for the human diet. Several health-promoting effects have been associated with kiwifruits, such as anticancer and antimicrobial activity (12), protection against DNA damage (13), cardiovascular protective properties (14), and antidiabetic function (15). Kiwifruit is a plant food displaying some molecular peculiarities. In fact, kiwifruit also contains protein molecules not reported in other fruits so far. For

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instance, the nutraceutical peptide kissper (16) has been detected in green kiwifruit only. Kissper is a 39-residue peptide deriving from the proteolytic processing of the precursor kiwellin, a two-domain protein (17) found in very high amounts in kiwifruit (18, 19). The protease actinidin 7catalyzes the kiwellin processing, thus producing KiTH and kissper, corresponding to the C-terminal and N-terminal domains, respectively (20). The kissper showed antioxidant and anti-inflammatory activities in experiments carried out in human models such as cultured intestinal cells and ex vivo colonic tissues from subjects suffering from Crohn's disease (21). However, the effect of kissper in Crohn's disease was impressive and we hypothesized it might work also in more site of the gut and possibly also in a different type of inflammation. As matter of fact, the kissper, like other dietary components, may exert some effects on inflammation in the gut by a nonspecific mechanism. Other substances (cranberry, for example) have a similar nonspecific effect (9–16). The hypothesis is made that a modification of nutrition, adding a nutraceutical food, may be useful in any gut inflammatory condition, such as inflammatory bowel disease (IBD) and celiac disease.

In this study, we aimed to investigate the effect of the kiwifruit peptide kissper in controlling the inflammation in CeD intestinal mucosa. For this purpose, the capacity of kissper to counteract the gluten-related inflammation was investigated in duodenal mucosa from CeD patients and non-CeD patients by immunodetection of the level of specific molecular markers of inflammation.

## Materials and methods

### Kissper purification from green kiwifruit

Kissper was purified from green kiwifruits (*Actinidia deliciosa*) available on the market. Several batches were purchased, and the concentration of this peptide was analyzed in each one. A fruit batch containing a significant amount of kissper was selected and used for its purification, which was obtained following the already reported procedure (16) with a few modifications. Briefly, 300 g of kiwifruits was homogenized by a household blender. The centrifugate supernatant was dialyzed against 10 mM Tris-HCl, pH 8.0, and loaded onto a DE52 (Whatman, Brentford, UK) column (2.5 × 18 cm) equilibrated in the same buffer. The column was eluted with increasing ionic strength using 0.5 M NaCl in the equilibrating buffer and aliquots (0.2 ml) of the collected 3-ml fractions were analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC) (16).

The fractions containing kissper were dialyzed against 10 mM Tris-HCl, pH 8.0, and then the peptide was further purified by fast protein liquid chromatography (FPLC) (Amersham-Pharmacia, Milan, Italy) carried out on a Mono-Q HR 10/10 column (Amersham-Pharmacia), equilibrated in the same buffer. The column was eluted with a linear gradient from zero to 0.3 M NaCl, at a flow rate of 3 ml/min, recording the absorbance at 280 nm, collecting 3-ml fractions (16). The fractions from Mono-Q were analyzed by RP-HPLC by loading 0.1 ml of fractions showing

absorbance at 280 nm, and those containing kissper were dialyzed against 2 mM NaCl and concentrated to 1 mg/ml by ultrafiltration on Ultracel 3K Amicon Ultrafilters (Millipore, Carrigtwohill, Ireland).

### Analysis of the kissper preparation purity

The peptide preparation was considered pure and therefore suitable for the functional studies when it appeared free of any contaminant molecule. The purity of the peptide preparation was analyzed by RP-HPLC and N-terminal amino acid sequencing. The RP-HPLC separation showed a single peak in the chromatographic profile, thus indicating the absence of contaminant molecules. Amino acid sequencing of the N-terminal region of the purified kissper was performed with an Applied Biosystems Procise 492 automatic sequencer (Applied Biosystems, Foster City, CA), equipped with online detection of phenylthiohydantoin amino acids (16). Briefly, a peptide solution at the concentration of 1 mg/ml was diluted 1:1 with distilled water. Four microliters, containing 2 µg of kissper, was picked up from the 0.5 mg/ml peptide solution and loaded on the automated amino acid sequencer. Ten sequencing cycles, based on Edman degradation chemistry, were run and the following amino acid sequence was obtained: ISSCNGPCRD. This sequence allowed the unequivocal identification of kissper. In fact, the homology search performed using the sequence ISSCNGPCRD and the BLAST algorithm, on the expasy website [www.expasy.org](http://www.expasy.org), showed that the only kiwifruit protein molecule having this N-terminal sequence was kissper. In addition, the results obtained from the automated sequencing showed the absence of contaminant amino acids. Hence, they also confirmed the purity of kissper preparation.

### Patients and ex vivo organ culture

After informed consent, 10 patients with suspected celiac disease (positive serology) on a gluten-containing diet (25 ( $m \pm 7.039$ ), range 16–41 years) (CeD group) and 4 patients complaining gastro-oesophageal reflux (24.2 ( $m \pm 4.85$ ), range 18–29 years) (non-CeD group) underwent endoscopy and multiple biopsies in the second and third part of the duodenum. After excision, each biopsy was placed in a tube with NaCl 0.9% and then analyzed using a dissecting microscope for a correct orientation. Five duodenal fragments were collected from each patient. Two fragments were oriented on Whatmann paper for routine histology. The remaining 3 fragments were placed in the ice-cold tissue-culture medium within 20 minutes. After that, each biopsy was sliced into 3 approximately equal sized pieces using a scalpel. The piece was placed on a stainless-steel mesh, positioned over the central well of an organ culture dish (Falcon, USA) with the villous surface of the biopsies uppermost. The well was then filled with culture medium at 37 °C so as to just reach the cut surface of the biopsies. Twenty milliliters of culture medium consisted of DMEM F12 (16 ml), fetal calf serum (3 ml), penicillin at 50,000 IU, and streptomycin at 5000 IU. The dishes were placed in a sterile

anaerobic jar, which was gassed with 95% oxygen/5% carbon dioxide for 30 minutes before being sealed and incubated at 37°C (22, 23). Biopsies were incubated in medium with the addition of bovine albumin solubilized in 2 mM NaCl (10 µg/ml), P31-43 (1 mg/ml), which is a non-immunodominant peptide derived from digestion of the gliadin (known to induce small intestine damage in celiac disease) and with and without the addition of kissper at a final concentration of (10 µg/ml). After 24 hours in culture the tissue was snap frozen in liquid nitrogen and stored at -80°C. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted by the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Campania Sud (n\_63 r.p.s.o. 19/10/2015).

### **Immunostaining of tissues**

Five-micrometer-thick cryostat mucosal sections were fixed in acetone for 10 minutes. The sections were incubated individually for 2 hours at room temperature with the following antibodies: human tissue transglutaminase 2 (TG2, clone + CUB7402; NeoMarkers, Fremont, CA) and HLA-DR (BD, Becton, Dickinson, Milan, Italy). Antigen expression and distribution were visualized using a donkey anti-rabbit IgG conjugated to Alexa Fluor 488 for 60 minutes at room temperature. Subsequently, tissue sections of culture explants were incubated for 5 minutes with DAPI (Invitrogen, diluted 1:1000 with deionized water) and then mounted with Mounting media (using glycerol and phosphate-buffered saline [PBS] at 5:1 v/v ratio).

Data were analyzed under fluorescence examination using an epifluorescent Leica DM4000B microscope. Images were captured using a Leica DFC300 FX camera (obj. 20×) and imaging software. The filters used are Filter cube A: MicFilt: A; Exci: UV, Excitation Filter BP 340-380; Dichromatic Mirror: 400; Suppression Filter LP425. Filter cube I3: MicFilt I3; Exci: blue; Excitation Filter BP 450-490; Dichromatic Mirror 510; Suppression Filter LP515. In our experiments, we used DAPI, a blue-fluorescent dye with excitation ideally suited to 405 nm and Alexa Fluor 488 for TG2 and HLA-DR; this dye is a bright, green-fluorescent dye with excitation ideally suited to the 488-nm laser line.

### **Evaluation of immunofluorescence intensity**

The intensity of immunofluorescence was scored from 0 to 3 for each one of the markers analyzed as reported (24). Intensity score of HLA-DR was based on the cellular localization of its expression (0, no expression; 1, expression in the basolateral membrane; 2, expression in the basolateral membrane and cytoplasmic compartment; 3, expression in basolateral membrane, cytoplasmic compartment, and apical membrane). Therefore, the higher the score, the higher is the expression (and quantity) of the marker examined. The evaluation of HLA-DR expression focused on villous enterocytes at time point 3 hours and on crypt enterocytes at time point 24 hours, respectively. The intensity score of TG2 was

based on the expression of this marker below the basement membrane (along with the villous or along the crypt or around mucosal vessels). For each of the markers, the challenge was considered positive when the intensity score in gliadin-exposed samples was at least 1 unit higher than the intensity score in blank samples. For example, the challenge was considered positive if the intensity score was 0 in the blank sample and 1 or higher in the gliadin-exposed sample. The intensity score was graded 1 in the blank sample and 2 or higher in the gliadin-exposed sample. On the other hand, the challenge was considered negative if the intensity score was 0 in the blank samples and the gliadin-exposed sample, as well as if the intensity score was 1 in the blank sample and 1 or lower in the gliadin-exposed sample, and so on (25). The evaluation of immune staining was independently analyzed in a blinded manner by two observers (IR and CDG); the results were compared afterward (24).

### **Western blotting analysis**

The intestinal explants specimen were lysed in buffer (50 mmol/L Tris-HCl, pH 8.0), with 150 mmol/L NaCl, 0.1% sodium dodecyl sulfate (SDS), 1% NP40, supplemented with protease and phosphatase inhibitors (CalbioChem). The proteins present in the supernatants were quantified with the Bradford method assay (Biorad). Samples of 40 µg total proteins from intestinal biopsy preparation were applied to 10% sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto nitrocellulose membrane (Biorad). Blotting membranes were incubated with blocking solution with 5% nonfat dried milk powder dissolved in Tris-buffered saline Tween-20 (TBST) buffer (pH 7.5, 10 mmol/L Tris-HCl, 150 mmol/L NaCl, and 0.1% Tween20) for 1 hour at room temperature, washed 3 times, and then incubated with mouse monoclonal antiTG2, (Novus Biologicals, cat. NB100-79920; 1:5000 dilution) and COX-2 (Life Technologies, 1:1000) in TBST, overnight, at 4°C. After 3 washes with TBST buffer, the membranes were reincubated for 1 hour with horseradish peroxidase-linked goat anti-mouse secondary antibody (Immunoreagents, Inc., 1:2000 dilution). Internal control was carried out using  $\beta$ -actin rabbit antibody (ABM, catalogue number Y080025; 1:1000 dilution). The membranes were then processed by using Chemidoc (Bio-rad, Laboratories S.r.l., Segrate, MI) and treated with enhanced chemiluminescence Western blotting detection reagents (Clarity Western ECL Substrate, Biorad, catalogue number 170-5060). The films were scanned, and the band's densitometry analysis was performed by using the ImageJ software for the relative level of the protein quantification (Image J 1.4r free Internet version).

### **Enzyme-linked immunosorbent assay (ELISA)**

IL-15 was measured using the Boster Biological Technology (Abingdon, UK) by the manufacturer's instructions. TGF- $\beta$ 1 was measured using the Elabscience kit (Houston, TX). Protein concentrations of whole-culture supernatants were

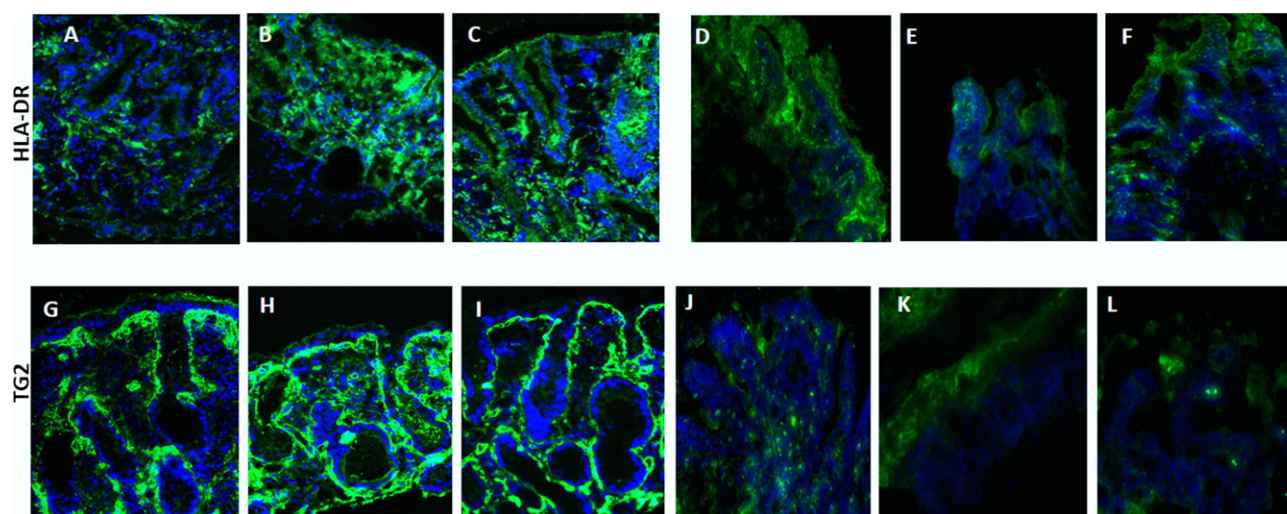


**Figure 1.** HLA-DR expression in the intestinal culture explant (immunofluorescence assay) from 1 representative experiment of CeD patient and 1 non-CeD patient. (A, B, C) CeD patient: (A) HLA-DR expression (green) (medium condition); (B) HLA-DR expression in the presence of P31-43; (C) HLA-DR expression after pretreatment with kissper at 10  $\mu$ g/ml and P31-43. DAPI nuclear counterstains, blue. (D-E-F, non CeD patient): (D) HLA-DR expression (green) (medium condition) in non CeD patient; (E) HLA-DR expression in the presence of P31-43; (F) HLA-DR expression after pretreatment with kissper at 10  $\mu$ g/ml and P31-43. DAPI Nuclear Counterstains, blue. (G, H, I) CeD patient: (G) TG2 expression (green) (medium condition); (H) TG2 expression in the presence of P31-43; (I) TG2 expression after pretreatment with kissper at 10  $\mu$ g/ml and P31-43. DAPI nuclear counterstains, blue. (J, K, L) Non-CeD patient: (J) TG2 expression (green), in medium condition; (K) TG2 expression in the presence of P31-43; (L) TG2 expression after pretreatment with kissper at 10 mg/ml and P31-43. DAPI nuclear counterstains, blue.

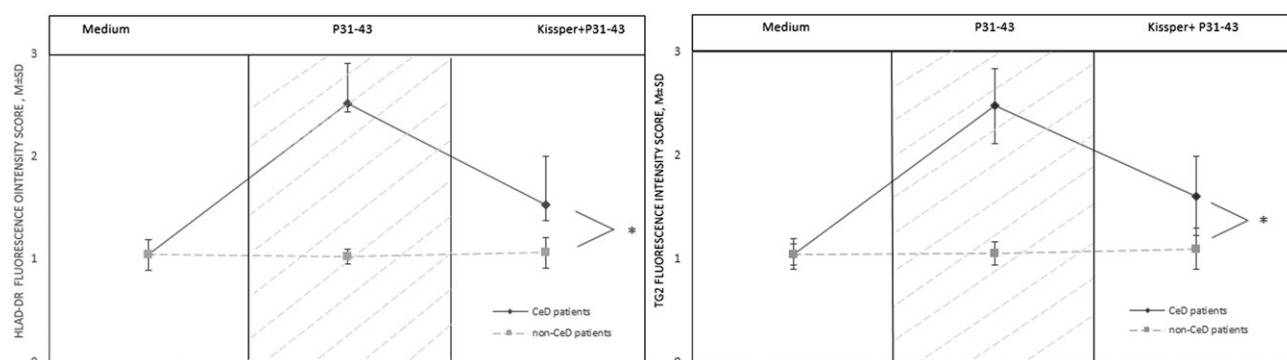


**Figure 2.** Fluorescence intensity scores of HLA-DR and TG2 expression in CeD patients and non-CeD patients during medium condition, treatment with P31-43 and pretreatment with kissper at 10 mg/ml and P31-43. The intensity scores of fluorescence expression for HLA-DR and TG2 during mucosal duodenal challenge with P31-43 significantly decrease with the preincubation with kissper in CeD patients compared to non-CeD patients (repeated-measure ANCOVA, with intensity scores of duodenal mucosa incubated with the medium only as covariate,  $p < 0.01$ ). Values are expressed as mean  $\pm$  SD; asterisk indicates  $p$  values were considered statistically significant when lower than 0.05.

measured using the Tecan Sunrise RC. Proteins levels were normalized to standard protein concentrations.

TGF- $\beta$ 1 assay characteristics included reactivity: human detection; range: 31.25–2000 pg/ml; and sensitivity: 18.75 pg/ml. The optical density (OD) value of each well was determined at once with a microplate reader set to 450 nm. TMB reaction followed the incubation at 37 °C for 15–20 minutes (Bradford assay). The IL-15 assay characteristics were reactivity of human detection, range 15.6 pg/ml–1000 pg/ml, and sensitivity <3 pg/ml. TMB reaction followed the incubation at 37 °C for 15–20 minutes. Then the OD absorbance at 450 nm in a microplate was read (spectrophotometric detection.).

### Statistical analysis

Categorical and continuous variables were expressed as frequency and mean  $\pm$  SD, respectively. The comparisons between CeD patient and non-CeD patient groups were

assessed using different inflammatory markers during the P31-43 treatment and P31-43 + kissper treatment of duodenal mucosa using a repeated-measure analysis of covariance (ANCOVA) model (26). The value of the intensity score obtained from duodenal mucosa incubated with medium only was used as the covariate to account for the potential effects of differences between the two groups (25). The  $p$  values were considered statistically significant when lower than 0.05.

## Results

### Evaluation of HLA-DR and TG2 expression in the intestinal tissues of CeD and non-CeD patients

Experiments carried out on CeD patients' duodenal mucosa showed that the expression of HLA-DR, highlighted by the green fluorescence, increased after the treatment with the gliadin peptide P31-43 (Figure 1B), compared to CeD patients' duodenal mucosa incubated with the medium only

**Table 1.** Intensity Scores of HLA-DR, TG2, IL-15, and TGF-  $\beta$ 1 in Medium Condition, After P31-43 and P31-43 + Kissper.

| CeD patients                  |                   |                    |                   | Non-CeD patients       |                      |                    |                     |
|-------------------------------|-------------------|--------------------|-------------------|------------------------|----------------------|--------------------|---------------------|
| HLA-DR (n = 10)               | Medium            | P31-43             | P31-43 + kissper  | HLA-DR (n = 4)         | Medium               | P31-43             | P31-43 + kissper    |
|                               | 1.05 $\pm$ 0.15   | 2.52 $\pm$ 0.39    | 1.53 $\pm$ 0.48   |                        | 1.05 $\pm$ 0.15      | 1.03 $\pm$ 0.075   | 1.07 $\pm$ 0.15     |
| TG2 (n = 10)                  | 1.05 $\pm$ 0.15   | 2.48 $\pm$ 0.36    | 1.61 $\pm$ 0.38   | TG2 (n = 4)            | 1.05 $\pm$ 0.10      | 1.055 $\pm$ 0.11   | 1.1 $\pm$ 0.20      |
| IL-15 (n = 8). pg/ml          | 2.72 $\pm$ 1.41   | 2.78 $\pm$ 1.25    | 2.60 $\pm$ 0.82   | IL-15 (n = 3)          | 0.6 $\pm$ 0.00       | 0.6 $\pm$ 0.00     | 0.6 $\pm$ 0.00      |
| TGF- $\beta$ 1 (n = 6). pg/ml | 2021 $\pm$ 193.00 | 1929.3 $\pm$ 188.8 | 2033 $\pm$ 196.05 | TGF- $\beta$ 1 (n = 4) | 1877.25 $\pm$ 159.91 | 2071.0 $\pm$ 201.0 | 2002.25 $\pm$ 108.7 |

Note. Data are expressed as mean  $\pm$  SD.

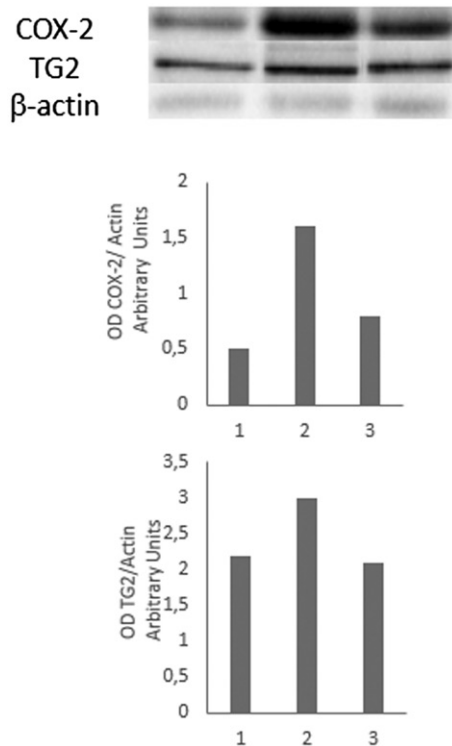


**Figure 3.** Western blot analysis of COX-2 and TG2 in intestinal culture explants: (1) Stimulation only with the culture medium; (2) stimulation with P31-43; (3) pretreatment with kissper and P31-43 stimulation. Representative data from a single CeD patient.

(Figure 1A). The HLA-DR expression induced by the gluten challenge with P31-43 strongly decreased when kissper (10  $\mu$ g/ml) was added. Specifically, Figure 1C shows that the green fluorescence intensity decreased in villous enterocytes and crypt epithelial cells.

Similarly, the expression of TG2, highlighted by the green fluorescence, increased after treatment with the gliadin peptide P31-43 (Figure 1H), compared to CeD patients' duodenal mucosa incubated with the medium only (Figure 1G). Figure 1I shows that preincubation with kissper (10  $\mu$ g/ml) caused a reduction of duodenal mucosa TG2 expression. In particular, a high increase in staining of TG2 after P31-43 stimulation was seen within the subepithelial region of the lamina propria, extracellular matrix, and enterocytes, and this staining decreased after the preincubation with kissper. Figure 1 (parts D, E, F: HLA-DR; and parts J, K, L: TG2) shows the HLA-DR and TG2 expression in non-CeD patients in which we did not see differences between duodenal mucosa treated with P31-43 and the duodenal mucosa treated with P31-43 after preincubation with kissper. We added also the treatment with kissper alone and albumin, which are presented as supplemental material.

See the Materials and methods section for the score interpretation.

### Comparisons of HLA-DR and TG2 expression between groups

In the comparisons between groups, the intensity scores of fluorescence expression for HLA-DR and TG2 during mucosal duodenal challenge with P31-43 significantly decrease with the preincubation with kissper compared to non-CeD patients (repeated-measure ANCOVA  $p < 0.01$ ), shown in Figure 2 (A and B) and Table 1.

### Evaluation of TGF- $\beta$ 1 and IL-15 levels in the supernatants of intestinal tissues from CeD and non-CeD patients

Experiments carried out on CeD patients' duodenal mucosa showed that there were no differences in TGF- $\beta$ 1 and IL-15 concentration levels of supernatants among CeD patients' duodenal mucosa incubated with the medium only, CeD patients' duodenal mucosa challenged with P31-43, and those challenged with P31-43 preincubated with kissper (10  $\mu$ g/ml).

Similarly, non-CeD patients did not show any differences in TGF- $\beta$ 1 and IL-15 concentration in duodenal mucosa incubated with medium only, treated with P31-43, and treated with P31-43 after preincubation with kissper.

### Comparisons of IL-15 and TGF- $\beta$ 1 levels between groups

In the comparison between groups the intensity score of IL 15 is significantly higher in CeD patients compared to non-CeD patients during mucosal duodenal challenge with P31-43 and during the preincubation with kissper (repeated-measure ANCOVA  $p < 0.04$ , with a significant difference also among groups for medium only,  $p < 0.001$ ). On the converse, no difference was found between groups in the level of TGF- $\beta$ 1 in CeD patients compared to non-CeD patients during mucosal duodenal challenge with P31-43 and during the preincubation with kissper (repeated-measure ANCOVA  $p = 0.07$ , with a significant difference among groups for medium only  $p < 0.01$ ).

### Evaluation of COX-2 and TG2 expression by Western blot

Western blot analysis of TG2 and COX-2 was conducted in 3 of the 10 patients examined in our study. Patient 1 showed a reduction of 19% of TG2 and no significant

reduction in the COX-2 expression with kissper pretreatment in comparison with treatment with P31-43. Patient 2 showed a reduction of 30% of TG2 and a reduction of 75% of COX-2 expression with kissper pretreatment compared to treatment with P31-43 (Figure 3). Patient 3 showed a reduction of 40% of TG2 and a reduction of 54% of COX-2 in the presence of kissper pretreatment in comparison with P31-43 treatment.

## Discussion

A gluten-free diet represents, at this moment, the best treatment for patients suffering from CeD (27). Most CeD subjects find relief from their symptoms and their intestine begins to heal within a few weeks after the application of the gluten-free diet. Nevertheless, recovery speed varies among patients, and sometimes a long time, even years, is required to achieve healing. It can also happen that the intestine never fully recovers from the damage due to CeD even if the patient remains on a strict gluten-free diet. However, we know that many dietary components may contribute to improve the intestinal health and speed its healing, counteracting the inflammatory processes. For example, kiwifruit and some other fruits and vegetables have shown an effect on the immunological mechanisms (28).

The study here reported suggests that kissper, a peptide naturally occurring in the green kiwifruit, is a nutraceutical molecule that can contribute to reducing inflammation in the mucosa of CeD subjects. The data were obtained using a model representing a good approximation to *in vivo* studies, consisting of cultured biopsy tissues where all the anatomical connections are retained, and all the cell types interact in their natural environment. In these experimental conditions, kissper showed the ability to control the inflammation induced by a fragment of alfa2-gliadin, the so-called “toxic” peptide P31-43, which has been reported to induce the innate immune response in celiac subjects (29). In fact, the gluten-induced overexpression of specific markers, such as HLA-DR (30, 31), TG2 (32), and COX-2 (33), was counteracted by the pretreatment with kissper. The results reported in this article highlight that kissper can affect the function of this receptor, inducing a significant decrease in its expression of HLA-DR and TG2 in villous enterocytes and crypt epithelial cells. HLA-DR is an MHC class II cell surface receptor consisting of an alpha chain and a beta chain, both anchored in the membrane, and binds and presents peptides derived from extracellular proteins (34). COX-2 is an enzyme produced by monocytes and macrophages that increase in the inflammatory conditions (35). These proteins and the TG2 enzyme, localized in the mucosa, increase. Two different methodologies, one immunochemical and the other immunohistochemical, were applied to the investigation of the kissper effect on the TG2 concentration in the tissue treated with P31-43. Both the methodologies highlighted a decrease of this enzyme responsible for the deamidation and crosslinking of the gliadin fragments that activate the immune response in celiac disease. The pretreatment with kissper caused in all patients a significant reduction of HLA-

DR and TG2 (36). However, we failed to demonstrate the changes in TGF- $\beta$ 1 and reducing IL-15 release in the gluten challenge of CeD mucosa, likely due to the small number of samples (37). Moreover, variable results were obtained for the expression of the inflammation marker COX-2 after stimulation with P31-43, which in 1 patient showed a 75% reduction.

Altogether, our data indicate that kissper may exert a protective role, eliciting a “repair response mechanism” by the reduction of proinflammatory proteins, implicated in CeD. Different bioactive molecules have the potential capability to regulate the chronic intestinal inflammation by inducing the reduction of inflammatory mediators. In fact, they can affect cell signaling and gene expression, causing a decrease of the damaging effects of oxidants and improving the gut barrier function. Vitamins, such as vitamin C and vitamin E contained in plant foods, can also exert antioxidant activity and have a positive effect on the immune response dysregulation associated with several pathologies by acting on the leukocyte function and lymphocyte (38, 39). Therefore, we hypothesized that at least some health-promoting activities of kiwifruits might be associated with this molecule. Kissper displays pH-dependent and voltage-gated pore-forming activity, with anion selectivity and channeling in artificial planar lipid membranes having a composition similar to that of the human intestine (14). In conclusion, kissper should be seen as a molecule with a potential role favoring the recovery of the histologically and functionally damaged intestine in CeD patient. This food molecule is likely resistant to the gastrointestinal proteases (19), although to our knowledge it is not known for how long the kissper resists in the intestine. However, if kissper will have a future as “protection” against gluten damage, we believe that it would be possible to administer kissper in a pill that gradually releases it on the mucosal surface. The data here described prompt further investigations to understand whether the consumption of kiwifruit or the integration of diet with an appropriate amount of kissper can control the symptoms of CeD subjects due to the ingestion of low amounts of gluten present as contamination of foods. Also, it is of great interest to investigate whether the kiwifruit peptide kissper has the characteristics of a “pharmacological molecule” useful for the treatment of inflammatory gut pathologies.

## Conclusions

Our results point to the identification of a potential therapeutic agent, a kiwifruit peptide, to control inflammation in CeD. Our preliminary results suggest that kissper has in CeD a potential role as an anti-inflammatory molecule in the presence of the toxic gliadin peptide P31-43, due to the modulation of the levels of different mediators of inflammation. Obviously, other studies are required to demonstrate that kiwi itself, or some other kiwifruit extracts like the kissper peptide, may be a valuable support in the diet of CeD patients. Our data support the idea that kissper may perhaps protect from trace gluten contamination of the diet.

## Conflict of interest

The authors declare no conflicts of interest.

## Authors' contributions

C.C. and I.R. conceived and designed the experiments and wrote the article. I.R. and C.D.G. performed the experiments, analyzed data and drafted the article. P.I. provided the biopsies samples and statistical analysis. I.G. and M.C. provided the kissper peptide, helped with the study design, and drafted the article. All authors read and approved the final article.

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## References

- Catassi C, Gatti S, Lionetti E. World perspective and celiac disease epidemiology. *Dig Dis*. 2015;33(2):141–6. doi: [10.1159/000369518](#)
- Lundin KE, Sollid L. M. Gliadin peptide specific intestinal T cells in coeliac disease. *Gut*. 2003;52(2):162.
- Sollid LM. Coeliac disease: dissecting a complex inflammatory disorder. *Nat Rev Immunol*. 2002;2(9):647–55. doi: [10.1038/nri885](#)
- Maiuri L, Ciacci C, Ricciardelli I, Vacca L, Raia V, Rispo A, Griffin M, Issekutz T, Quarantino S, Londei M. Unexpected role of surface transglutaminase type II in celiac disease. *Gastroenterology*. 2005;129(5):1400–13.
- Maiuri L, Ciacci C, Ricciardelli I, Vacca L, Raia V, Auricchio S, Picard J, Osman M, Quarantino S, Londei M. Association between innate response to gliadin and activation of pathogenic T cells in coeliac disease. *Lancet*. 2003;362(9377):30–7. doi: [10.1016/S0140-6736\(03\)13803-2](#)
- Abadie V, Jabri B. IL-15: a central regulator of celiac disease immunopathology. *Immunol Rev*. 2014;260(1):221–34. doi: [10.1111/imr.12191](#)
- Pérez-Aisa A, Sopena F, Arceiz E, Ortego J, Sainz R, Lanás A. Effect of exogenous administration of transforming growth factor-beta and famotidine on the healing of duodenal ulcer under the impact of indomethacin. *Dig Liver Dis*. 2003;35(6):397–403.
- Kagnoff M. Mucosal inflammation in celiac disease: interleukin-15 meets transforming growth factor  $\beta$ -1. *Editorials Gastroenterology*. 2007;132(3):1174–76.
- Calder PC, Albers R, Antoine JM, Blum S, Bourdet-Sicard R, Ferns GA, Folkerts G, Friedmann PS, Frost GS, Guarner F, Løvik M. Inflammatory disease processes and interactions with nutrition. *BJN*. 2009;101(1):S1–S45. doi: [10.1017/S0007114509377867](#)
- Calder PC. Fatty acids and inflammation: the cutting edge between food and pharma. *Eur J Pharmacol*. 2011;668(1):S50–S8. doi: [10.1016/j.ejphar.2011.05.085](#)
- Giangrieco I, Proietti S, Moscatello S, Tuppo L, Battistelli A, La Cara F, Tamburrini M, Famiani F, Ciardiello MA. Influence of geographical location of orchards on green kiwifruit bioactive components. *J Agric Food Chem*. 2016;64(48):9172–9. doi: [10.1021/acs.jafc.6b03930](#)
- Motohashi N, Shirataki Y, Kawase M, Tani S, Sakagami H, Satoh K, Kurihara T, Nakashima H, Mucsi I, Varga A, Molnár J. Cancer prevention and therapy with kiwifruit in Chinese folklore medicine: a study of kiwifruit extracts. *J Ethnopharmacol*. 2002;81(3):357–64.
- Jung KA, Song TC, Han D, Kim IH, Kim YE, Lee CH. Cardiovascular protective properties of kiwifruit extracts in vitro. *Biol Pharm Bull*. 2005;28(9):1782–5.
- Collins AR, Harrington V, Drew J, Melvin R. Nutritional modulation of DNA repair in a human intervention study. *Carcinogenesis*. 2003;24(3):511–5.
- Abe D, Saito T, Kubo Y, Nakamura Y, Sekiya K. A fraction of unripe kiwi fruit extract regulates adipocyte differentiation and function in 3T3-L1 cells. *Biofactors*. 2010;36(1):NA–N9. doi: [10.1002/biof.70](#)
- Ciardiello MA, Meleleo D, Saviano G, Crescenzo R, Carratore V, Camardella L, Gallucci E, Micelli S, Tancredi T, Picone D, Tamburrini M. Kissper, a kiwi fruit peptide with channel-like activity: structural and functional features. *J Pept Sci*. 2008;14(6):742–54. doi: [10.1002/psc.992](#)
- Offermann LR, Giangrieco I, Perdue ML, Zuzzi S, Santoro M, Tamburrini M, Cosgrove DJ, Mari A, Ciardiello MA, Chruszcz M. Elusive structural, functional, and immunological features of Act d 5, the green Kiwifruit Kiwellin. *J Agric Food Chem*. 2015;63(29):6567–76. doi: [10.1021/acs.jafc.5b02159](#)
- Tamburrini M, Cerasuolo I, Carratore V, Stanzola AA, Zofra S, Romano L, Camardella L, Ciardiello MA. Kiwellin, a novel protein from kiwi fruit. Purification, biochemical characterization and identification as an allergen. *Protein J*. 2005;24(7–8):423–9. doi: [10.1007/s10930-005-7638-7](#)
- Ciardiello MA1, Giangrieco I, Tuppo L, Tamburrini M, Buccheri M, Palazzo P, Bernardi ML, Ferrara R, Mari A. Influence of the natural ripening stage, cold storage, and ethylene treatment on the protein and IgE-binding profiles of green and gold kiwi fruit extracts. *J Agric Food Chem*. 2009;57(4):1565–71. doi: [10.1021/jf802966n](#)
- Tuppo L, Giangrieco I, Palazzo P, Bernardi ML, Scala E, Carratore V, Tamburrini M, Mari A, Ciardiello MA. Kiwellin, a modular protein from green and gold kiwi fruits: evidence of in vivo and in vitro processing and IgE binding. *J Agric Food Chem*. 2008;56(10):3812–7. doi: [10.1021/jf703620m](#)
- Ciacci C, Russo I, Bucci C, Iovino P, Pellegrini L, Giangrieco I, Tamburrini M, Ciardiello MA. The kiwi fruit peptide kissper displays anti-inflammatory and anti-oxidant effects in in-vitro and ex-vivo human intestinal models. *Clin Exp Immunol*. 2014;175(3):476–84. doi: [10.1111/cei.12229](#)
- Fais S, Maiuri L, Pallone F, De Vincenzi M, De Ritis G, Troncone R, Auricchio S. Gliadin induced changes in the expression of MHC-class II antigens by human small intestinal epithelium. Organ culture studies with coeliac disease mucosa. *Gut*. 1992;33(4):472–5.
- Russo I, Zeppa P, Iovino P, Del Giorno C, Zingone F, Bucci C, Puziello A, Ciacci C. The culture of gut explants: a model to study the mucosal response. *J Immunol Methods*. 2016;438:1–10. doi: [10.1016/j.jim.2016.07.004](#)
- Maiuri L, Picarelli A, Boirivant M, Coletta S, Mazzilli MC, De Vincenzi M, Londei M, Auricchio S. Definition of the initial immunologic modifications upon in vitro gliadin challenge in the small intestine of celiac patients. *Gastroenterology*. 1996;110(5):1368–78.
- Tortora R, Russo I, De Palma GD, Luciani A, Rispo A, Zingone F, Iovino P, Capone P, Ciacci C. In vitro gliadin challenge: diagnostic accuracy and utility for the difficult diagnosis of celiac disease. *Am J Gastroenterol*. 2012;107(1):111–7. doi: [10.1038/ajg.2011.311](#)
- Cappello C, Tremolaterra F, Pascariello A, Ciacci C, Iovino P. A randomised clinical trial (RCT) of a symbiotic mixture in patients with irritable bowel syndrome (IBS): effects on symptoms, colonic transit and quality of life. *Int J Colorectal Dis*. 2013;28(3):349–58.

27. Zingone F, Capone P, Ciacci C. Celiac disease: alternatives to a gluten free diet. *WJGPT*. 2010;1(1):36–9. doi: [10.4292/wjgpt.v1.i1.36](https://doi.org/10.4292/wjgpt.v1.i1.36)
28. Skinner MA, Bentley-Hewitt K, Rosendale D, Naoko S, Pernthaner A. Effects of kiwifruit on innate and adaptive immunity and symptoms of upper respiratory tract infections. *Adv Food Nutr Res*. 2013;68:301–20. doi: [10.1016/B978-0-12-394294-4.00017-1](https://doi.org/10.1016/B978-0-12-394294-4.00017-1).
29. Barone MV, Troncone R, Auricchio S. Gliadin peptides as triggers of the proliferative and stress/innate immune response of the celiac small intestinal mucosa. *IJMS*. 2014;15(11):20518–37. doi: [10.3390/ijms151120518](https://doi.org/10.3390/ijms151120518).
30. Sollid LM, Thorsby E. HLA susceptibility genes in celiac disease: genetic mapping and role in pathogenesis. *Gastroenterology*. 1993;105(3):910–22.
31. Mazzarella G, Stefanile R, Camarca A, Giliberti P, Cosentini E, Marano C, Iaquinto G, Giardullo N, Auricchio S, Sette A, et al. Gliadin activates HLA class I restricted CD8+ T cells in celiac disease intestinal mucosa and induces the enterocyte apoptosis. *Gastroenterology*. 2008;134(4):1017–27. doi: [10.1053/j.gastro.2008.01.008](https://doi.org/10.1053/j.gastro.2008.01.008).
32. Luciani A, Villella VR, Vasaturo A, Giardino I, Pettoello-Mantovani M, Guido S, Cexus ON, Peake N, Londei M, Quarantino S, Maiuri L. Lysosomal accumulation of gliadin p3143 peptide induces oxidative stress and tissue transglutaminase-mediated PPARgamma downregulation in intestinal epithelial cells and coeliac mucosa. *Gut*. 2010;59(3):311–9. doi: [10.1136/gut.2009.183608](https://doi.org/10.1136/gut.2009.183608).
33. Kainulainen H, Rantala I, Collin P, Ruuska T, Päävärinne H, Halttunen T, Lindfors K, Kaukinen K, Mäki M. Blisters in the small intestinal mucosa of coeliac patients contain T cells positive for cyclooxygenase 2. *Gut*. 2002;50(1):84–9.
34. Wieczorek M, Abualrous ET, Sticht J, Álvaro-Benito M, Stolzenberg S, Noé F, Freund C. Major histocompatibility complex (MHC) class I and MHC class II proteins: conformational plasticity in antigen presentation. *Front Immunol*. 2017;17(8):292. doi: [10.3389/fimmu.2017.00292](https://doi.org/10.3389/fimmu.2017.00292).
35. Roy R, Polgar P, Wang Y, Goldstein RH, Taylor L, Kagan HM. Regulation of lysyl oxidase and cyclooxygenase expression in human lung fibroblasts: interactions among TGF-beta, IL-1 beta, and prostaglandin E. *J Cell Biochem*. 1996;62(3):411–7. doi: [10.1002/\(SICI\)1097-4644\(199609\)62:3%3C411::AID-JCB11%3E3.0.CO;2-L](https://doi.org/10.1002/(SICI)1097-4644(199609)62:3%3C411::AID-JCB11%3E3.0.CO;2-L)
36. Esposito C, Paparo F, Caputo I, Porta R, Salvati VM, Mazzarella G, Auricchio S, Troncone R. Expression and enzymatic activity of small intestinal tissue transglutaminase in celiac disease. *Am J Gastroenterology*. 2003;98(8):1813–20. doi: [10.1111/j.1572-0241.2003.07582.x](https://doi.org/10.1111/j.1572-0241.2003.07582.x)
37. Hansson T, Ulfgrén A-K, Lindroos E, Dannaeus A, Dahlbom I, Klareskog L. Transforming growth factor- (TGF-) and tissue transglutaminase expression in the small intestine in children with coeliac disease. *Scand J Immunol*. 2002;56(5):530–7. PMID: 12410804.
38. Sorice A, Guerriero E, Capone F, Colonna G, Castello G, Costantini S. Ascorbic acid: its role in immune system and chronic inflammation diseases. *Mini Rev Med Chem*. 2014;14(5):444–52.
39. Tahan G, Aytac E, Aytakin H, Gunduz F, Dogusoy G, Aydin S. Vitamin E has a dual effect of anti-inflammatory and antioxidant activities in acetic acid-induced ulcerative colitis in rats. *Can J Surg*. 2011;54(5):333–8. doi: [10.1503/cjs.013610](https://doi.org/10.1503/cjs.013610).