# Effect of eicosapentaenoic acid-derived prostaglandin $E_3$ on intestinal epithelial barrier function

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A B S T R A C T

Prostaglandins (PG) are inflammatory mediators derived from arachidonic or eicosapentaenoic acid giving rise to the 2-series or the 3-series prostanoids, respectively. Previously, we have observed that $PGE_2$ disrupts epithelial barrier function. Considering the beneficial effect of fish oil consumption in intestinal inflammatory processes, the aim of this study was to assess the role of $PGE_3$ on epithelial barrier function assessed from transepithelial electrical resistance and dextran fluxes in Caco-2 cells. The results indicate that $PGE_3$ increased paracellular permeability (PP) to the same extent as $PGE_2$, through the interaction with $EP_1$ and $EP_4$ receptors and with intracellular $Ca^{2+}$ and cAMP as the downstream targets. Moreover, we observed a redistribution of tight junction proteins, occludin and claudin-4. In conclusion, $PGE_3$ is able to increase PP thus leading to reconsider the role of $PGE_2$/$PGE_3$ ratio in the beneficial effects of dietary fish oil supplementation in the disruption of barrier function.

## 1. Introduction

Many mediators of inflammation including prostaglandins (PG), leukotrienes (LT), and other oxygenated derivatives are synthesized from arachidonic acid (AA), an omega-6 (ω-6) poly-unsaturated fatty acid (PUFA) highly abundant in all mammalian cells. PG are involved in numerous physiological and biochemical processes and altered production is associated with a variety of disorders, including acute and chronic inflammation and colon cancer [1].

Nutritional regulation of PG generation may be modulated by dietary enrichment with o-3 fatty acids present in fish oil such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA can be incorporated into cell membrane phospholipids [2] and can also act as a substrate for cylooxygenase (COX), giving rise to the 3-series prostanoids [3]. Although similar in structure and stability [4], mediators formed from EPA are believed not to be as potently inflammatory as those formed from AA [5].In addition to reducing the concentrations of the proinflammatory 2-series PG, increased consumption of o-3 PUFA also results in a 10- to 50-fold increase in 3-series PG, thus reducing the PGE2/PGE3 ratio [6]. Along these lines, fish oil supplementation of the human diet has been used as a preventive measure against a number of diseases including coronary heart disease, cancer, and inflammatory bowel disease (IBD) [7]. However, to the best of our knowledge, no studies directly comparing the effects of the two series of PG on intestinal cellular functions have been reported.

The gastrointestinal epithelium functions as a selective barrier that allows the absorption of nutrients, electrolytes and water but restricts the passage of larger, potentially toxic compounds into systemic circulation. This characteristic of the intestinal epithe-lium, which has been referred to as selective permeability, is maintained by three distinct adhesion systems: tight junctions (TJs), adherent junctions, and desmosomes. Of these, TJs are the most apical component and are the rate-limiting step for paracellular permeability (PP). In addition, TJs constitute the interface (fence) between the apical and basolateral membrane domains [8]. TJs are multiprotein complexes composed of transmembrane proteins associated with the cytoskeletal perijunctional ring of actin and myosin. They also contain cytosolic proteins involved in cell signal-ing and vesicle trafficking. Several transmembrane proteins of the junctional complex have been identified: occludin, the claudin family, tricellulin, crumbs, and junctional adhesion molecules, among others. These proteins are associated with a wide range of cytosolic proteins, of which zona occludens (ZO), i.e. ZO-1, ZO-2, ZO-3, AF6, and cingulin are described as forming the nexus with cytoskeletal proteins [9].

Epithelial barrier function can be modulated by a number of factors under physiological or pathophysiological conditions. Intestinal diseases are associated with disruption of epithelial barrier function, particularly IBD [10]. The levels of several eicosanoids, such as $PGE_2$, $PGD_2$, thromboxane $B_2$, 5-, 12- and 15-hydroxyeicosatetraenoic acid, and leukotriene (LT) $B_4$ were found to be elevated in the mucosa of IBD patients [11]. It has been reported that fish oil decreases colonic inflammation compared with an o-6 PUFA-rich diet, associated with a reduction in the production of AA-derived eicosanoids in IBD experimental models. Moreover, some benefits in human trials of fish oil consumption in IBD have been reported [12].

We recently observed that the addition of PGE2 to differen-tiated intestinal Caco-2 cells induces an increase in PP, an effect mediated by the interaction of PGE2 with EP1 and EP4 receptors [13]. The main objective of this study was to assess in Caco-2 cells whether the 3-series PG effects on epithelial barrier function could explain, at least in part, the beneficial effects of fish oil consumption in IBD.

**2. Methods and materials**

*2.1. Materials*

DMEM, trypsin, penicillin, and streptomycin were supplied by GIBCO (Paisley, Scotland). Non-essential amino acids, fetal bovine serum (FBS), bovine serum albumin (BSA), phosphate buffered saline (PBS), D-glucose, HEPES, Fura-2 acetoxymethyl ester (Fura-2-AM), fluorescein isothiocyanate-dextran (FD-4, average mole-cular weight 3000–5000), PGE2, PGE3, U73122, dantrolene, G ¨o6983, and phalloidin-tetramethylrhodamine B isothiocyanate (TRITC-phalloidin), 1-(5-iodonaphthalene-1-sulfonyl)-1H-hexa-hydro-1,4-diazepine hydrochloride (ML-7), along with other che-micals, were purchased from Sigma-Aldrich (St. Louis, MO). Tissue culture supplies, including Transwells, were obtained from Costar (Cambridge, MA). ONO-8711 and ONO-AE3-240 were kindly provided by Ono Pharmaceutical Co. Ltd. (Osaka, Japan); and AH23848, by Glaxo-Wellcome (Stevenage, UK).

## 2.2. *Cell culture*

Caco-2 cells were provided by the American Type Cell Collec-tion and cultured as previously described [14]. Cells (passages 53–65) were routinely grown in plastic flasks at a density of $5 \times 10^4$ cells/cm$^2$ and cultured in DMEM containing 4.5 g/L D-glucose and 2 mM L-glutamine, supplemented with 1% (v/v) non-essential amino acids, 10% (v/v) heat-inactivated FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a modified atmosphere of 5% $CO_2$ in air. Cells grown to approximately 80%confluence were released by trypsinization and subcultured at a density of $4 \times 10^5$ cells/cm$^2$ on polycarbonate filters with a pore size of 0.4 μm (Transwells, 12 mm diameter) for PP experiments and at a density of $5 \times 10^4$ cells/cm$^2$ in 12-well clusters for intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) determination, in 24-well clusters containing coverslips for immunofluorescent microscopy, or in 75 cm$^2$ flasks for cAMP determination. For all experiments the same PGs concentration and incubation period were assayed as previously described for $PGE_2$ [13]. The medium was replaced every three days and on the day before the experiment. Experiments were performed in cultures 19–21 days after seeding, once the cells had differentiated [14].

## 2.3. *Paracellular permeability*

PP was estimated from transepithelial electrical resistance (TER) and transepithelial FD-4 fluxes. After 3 h incubation with the PGs in DMEM in the apical and basolateral compartments, TER was then determined at 37 °C using a Millicell-ERS voltohmmeter (Millipore, Bedford, MA). The results were expressed as Ω cm$^2$ monolayer surface area. The resistance of the supporting membrane was subtracted from all readings before calculations.

After TER determination, 1 mg/mL of FD-4 was added to the apical compartment and cells were incubated for 1 h at 37 °C. After incubation, the basolateral medium was removed and fluores-cence was determined in a Fluorostar Optima (BMG Labtech, Germany) at excitation and emission wavelengths of 485 nm and 544 nm, respectively.

## 2.4. *Intracellular $Ca^{2+}$ concentration*

$[Ca^{2+}]_i$ was monitored using the selective fluorescent $Ca^{2+}$ indicator Fura 2-AM as previously described [13]. Cells grown in clusters were loaded with 25 μM Fura 2-AM in DMEM for 1 h at 37 °C. The preloaded monolayers were then washed in modified Krebs buffer (pH 7.4) at 37 °C and incubated for 1 h at 37 °C to allow Fura 2-AM de-esterification. The monolayers were washed again to ensure the removal of all unloaded indicator, and eicosanoids and inhibitors were added to the respective wells. The fluorescent signal was continuously monitored with excita-tion wavelengths of 340 and 380 nm and emission at 510 nm using a fluorescent microplate reader (Fluorostar Optima, BMG Labtech, Germany) before and after injection of the eicosanoids. Cells were maintained at 37 °C throughout the experiment. At the end of the incubation period, the maximum and minimum intracellular probe fluorescent signals were determined by the addition of cell lysis buffer and 20 mM EDTA in Krebs, respec-tively. $[Ca^{2+}]_i$ was calculated following Grynkiewicz et al. [15] from a 340/380 ratio using a dissociation constant of 224 nM.

## 2.5. *Intracellular cAMP concentration*

cAMP determination was performed using a competitive enzyme immunoassay (EIA) Kit (Cayman, Ann Arbor, MI) follow-ing the manufacturer's instructions. Briefly, cells maintained in flasks were incubated for 5, 15, and 30 min at 37 °C with a range of PGs concentrations. They were then incubated for 20 min at room temperature with 2.5 mL of 0.1 N HCl and harvested and homogenized. The homogenate was then centrifuged (1000 g, 10 min) and the supernatant was assayed following the acetyla-tion procedure (sensitivity 0.1 pM).

## 2.6. *NFκB activation*

Cytosolic IκB proteins bind to the NFκB/Rel transcription factor complex to maintain its inactive state. For NFκB to become activated, it must first dissociate from the inhibitor IκB, thereby enabling NFκB to translocate into the nucleus to modulate gene expression. This is induced by phosphorylation of IκB at Ser32 and Ser36 in response to various extracellular signals, including inflammatory cytokines, growth factors, and chemokines. There-fore, NFκB activation was evaluated by measuring total and phosphorylated IκB using a competitive EIA Kit (eBioscience, San Diego, CA) following the manufacturer's instructions. Briefly, cells grown in clusters were incubated for 5 and 15 min and 3 h at 37 °C with $PGE_2$ and $PGE_3$ (3 nM) and TNF-α (100 ng/mL) as a positive control.

## 2.7. *Immunofluorescent staining of TJ proteins*

Control or treated Caco-2 monolayers grown on coverslips were washed gently with PBS and fixed in iced methanol or 3% paraformaldehyde (PF) and 2% sucrose in 0.1 M PBS (pH 7.4) for 15 min at −20 °C or room temperature, respectively. Cells were washed twice for 5 min in 10 mM PBS containing 20 mM glycine (PBS–glycine) and then permeabilized with 0.2% (v/v) Triton X-100 for 10 min at room temperature. Cells were washed twice in PBS-glycine and blocked for 20 min in PBS–glycine containing 1%

BSA (incubation solution). Mouse monoclonal anti-occludin (1:500 dilution; Zymed, South San Francisco, CA), rabbit polyclonal anti-ZO-1 (1:250 dilution; Zymed) and mouse polyclonal anti-claudin-2 (1:250 dilution; Invitrogen, San Diego, CA) were used as primary antibodies. Cells were incubated with the primary antibodies for 1 h at 37 °C and washed twice in PBS–glycine for 5 min at room temperature. Monolayers were then incubated for 1 h at 37 °C with Alexa dye-conjugated secondary antibodies (1:500 dilution, Molecular Probes, Leiden, The Netherlands). Finally, cells were washed for 10 min at room temperature in PBS, mounted in Mowiol (Calbiochem, San Diego, CA) and examined under an immunofluorescent microscope (BX 41, Olympus Barcelona, Spain). Images were taken using a 60x (numerical aperture 1.25, phase 3, oil) Olympus UPlanFL N objective. To view the actin subapical ring and claudin-1 and -4, coverslips were fixed in PF or methanol, respectively, then permeabilized as described above and incubated with TRITC-phalloidin for 1 h at 37 °C (1:1000 dilution) or direct-labeled claudin (1:250 dilution).

### 2.8. Data analysis

The results are expressed as mean ± SE. Data were analyzed by one-way analysis of variance followed by Dunnett's *post hoc* test using SPSS® software (SPSS Inc. Chicago, IL, USA). $P < 0.05$ was considered to denote significance.

## 3. Results

Here we have examined the effect of $PGE_3$ – a COX metabolite derived from EPA – on PP using differentiated Caco-2 cell monolayers. As observed in Fig. 1A, the extent to which ω-3 prostanoid decreased TER values was comparable to that of $PGE_2$. Treatment of Caco-2 cell monolayers with $PGE_3$ in the presence of $EP_1$ and $EP_4$ receptor antagonists, (ONO-8711 and AH-23848, respectively [16,17]), resulted in the reversion of the TER values whereas the $EP_3$ antagonist (ONO-AE3-240 [18]) did not significantly modify the TER values in comparison to $PGE_3$ alone.

Moreover, we observed that the effect induced by $PGE_3$ also altered TJ permeability by increasing FD4 fluxes to a similar level as when $PGE_2$ was used (Fig. 1B). In agreement with the results for TER, the incubation of $EP_1$ and $EP_4$ antagonists led to a decrease in FD4 permeability induced by $PGE_3$. Treatment with $EP_3$ antagonist also modified this value but to a lesser extent and without significant differences in comparison to $PGE_3$.

The ability of Gö6983, a pan-PKC inhibitor [19], and ML-7, a myosin light chain kinase (MLCK) inhibitor, to prevent $PGE_3$-induced PP alteration was also assessed. The results in Fig. 1 show that both Gö6983 and ML-7 were able to revert the changes in the values of TER and FD4 fluxes induced by $PGE_3$ to control conditions.

As shown in Fig. 2A, the addition of $PGE_3$ to Caco-2 cultures resulted in a concentration-dependent increase in the levels of $[Ca^{2+}]_i$ as when $PGE_2$ was added. Furthermore, the presence of U73122, a phospholipase C (PLC) inhibitor [20], and dantrolene, an inhibitor of intracellular $Ca^{2+}$ released from the endoplasmic reticulum [21], prevented this increase in $[Ca^{2+}]_i$ (Fig. 2B). The changes in $[Ca^{2+}]_i$ induced by $PGE_3$ were also reverted when $Ca^{2+}$ was withdrawn from the incubation media (data not shown).

For the cAMP pathway, the results shown in Fig. 3 reveal that $PGE_2$ and $PGE_3$ were able to induce an increase in cAMP.

The phosphorylation of IκB allows NFκB to translocate into the nucleus to modulate gene expression. To assess whether NFκB is activated in cells treated with $PGE_3$, IκB phosphorylation was determined. The results show that neither ω-6 nor ω-3 prostaglandins were able to increase the amount of phosphorylated IκB compared to control conditions in any of the incubation periods assayed (5 min, 15 min, and 3 h), whereas TNF-α, which was used as a positive control, was able to significantly increase IκB phosphorylation (Fig. 4).

The contribution of TJ proteins and cytoskeletal actin to PP regulation by $PGE_3$ was also investigated. The results of TJ protein immunofluorescent staining show that in control conditions occludin and ZO-1 are located mainly at the cell border. The

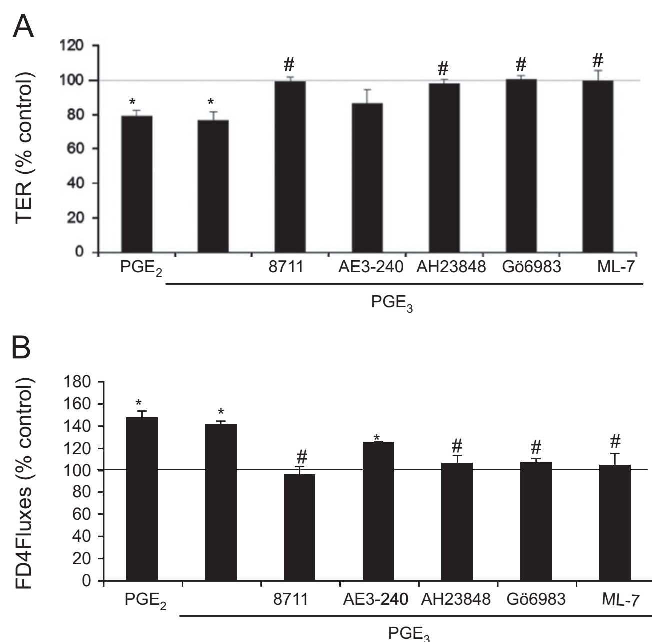


**Fig. 1.** Effect of $PGE_2$ and $PGE_3$ on epithelial barrier function TER (A) and FD-4 fluxes (B) were determined in differentiated Caco-2 cell monolayers, as described in "Materials and Methods". Cells were incubated for 3 h with PGs (3 nM) and $PGE_3$ plus ONO-8711 (250 nM), plus ONO-AE3-240 (2 nM), plus AH-23848 (200 nM), plus Gö6983 (1 μM), or plus ML7 (0.05 μM), in the apical and basolateral compartments. Results are expressed as the percentage of FD-4 fluxes and TER values obtained in control conditions (0.31 ± 0.02 ng/μL and 1689.69 ± 59.87 Ω cm$^2$, respectively). Data are means ± SE of $n$ = 6–8 experiments. $^*P < 0.05$ vs. control conditions. $^\#P < 0.05$ vs. $PGE_3$.

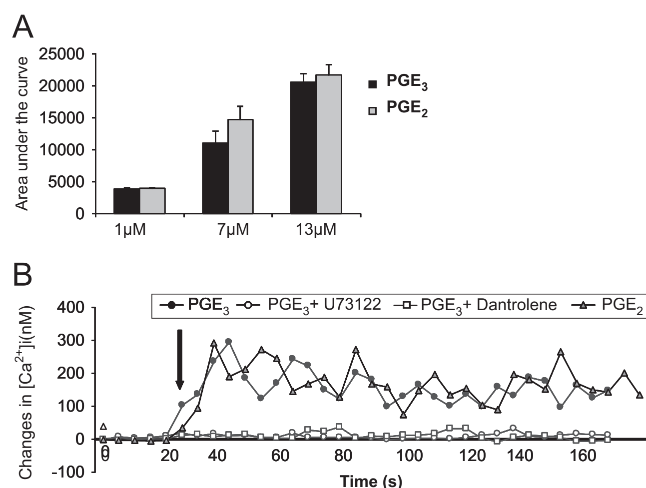


**Fig. 2.** Changes in $[Ca^{2+}]_i$ induced by $PGE_2$ and $PGE_3$. $[Ca^{2+}]_i$ were determined in differentiated Caco-2 cell monolayers using Fura-2 AM, as described in "Materials and Methods". (A) Cells were incubated for 180 s in the presence of PGs at different concentrations. Results are expressed as area under the curve (AUC). Data are means ± SE of $n$ = 3 experiments. (B) Cells were incubated in the presence of PGs (13 μM) and $PGE_3$ plus U73122 (0.1 μM) or plus dantrolene (50 μM). The arrow indicates the injection of PGs. Inhibitors were pre-incubated for 30 min. Each plot corresponds to a representative profile obtained for $n$ = 3 experiments.

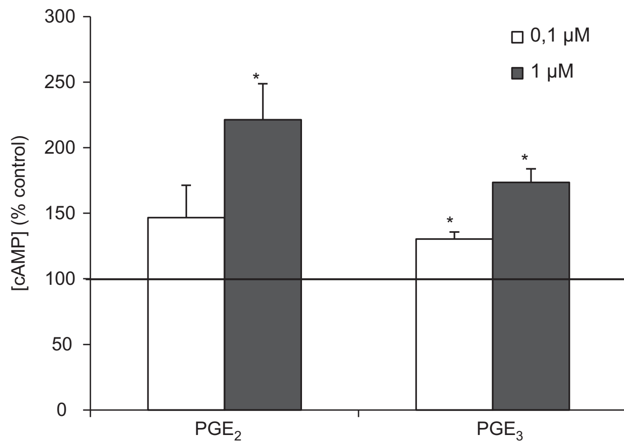


**Fig. 3.** Effect of $PGE_2$ and $PGE_3$ on intracellular cAMP concentration. cAMP was determined in differentiated Caco-2 cell, as described in "Materials and Methods". Cells were incubated for 5 min with $PGE_3$ and $PGE_2$ at two concentrations (0.1 μM, white bars and 1 μM black bars). Results are expressed as the percentage of cAMP values obtained in control conditions (23.54 ± 1.53 nM). The data are means ± SE of $n$=3–5 experiments. $^{*}P<0.05$ vs. control conditions.

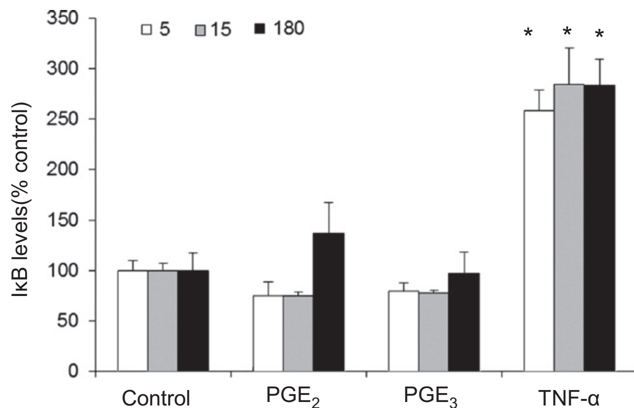


**Fig. 4.** Effect of $PGE_2$ and $PGE_3$ on phosphorylated IκB levels. Phosphorylated IκB levels were determined in differentiated Caco-2 cell monolayers as described in "Materials and Methods". Cells were incubated for 5 min, 15 min, or 3 h in the presence of the $PGE_3$ and $PGE_2$ (3 nM) or TNF-α (100 ng/mL) as a positive control. The results are expressed as the percentage with respect to control conditions. Data are mean ± SE of $n$=4–6 experiments. $^{*}P<0.05$ vs. control conditions.

treatment with $PGE_3$ resulted in a redistribution of occludin with adjacent diffuse intracellular staining and granular appearance (Fig. 5). No effect on ZO-1 location was observed (data not shown). The morphological assessment of subapical actin showed characteristic perijunctional rings in the control monolayers. The treatment with $PGE_3$ induced a complete disorganization of the F-actin belt. As seen in the control monolayers, claudin-4 was predominantly present at TJs and a weak cytoplasmic localization was also observed (Fig. 6). However, in response to either $PGE_2$ or $PGE_3$, claudin-4 markedly dissociated from the TJ to form protein clumps whereas the localization of claudin-1 and claudin-2 was not affected (data not shown) in neither of the PGs. Effects of $PGE_3$ on occludin and claudin-4 were partially prevented by $EP_1$ and $EP_4$ antagonists (ONO-8711 and AH-23848) as well as MLCK inhibitor (ML7) (Figs. 5 and 6).

## 4. Discussion and conclusions

The disruption of the intestinal TJ barrier results in an increase in paracellular permeability and is now believed to be involved in

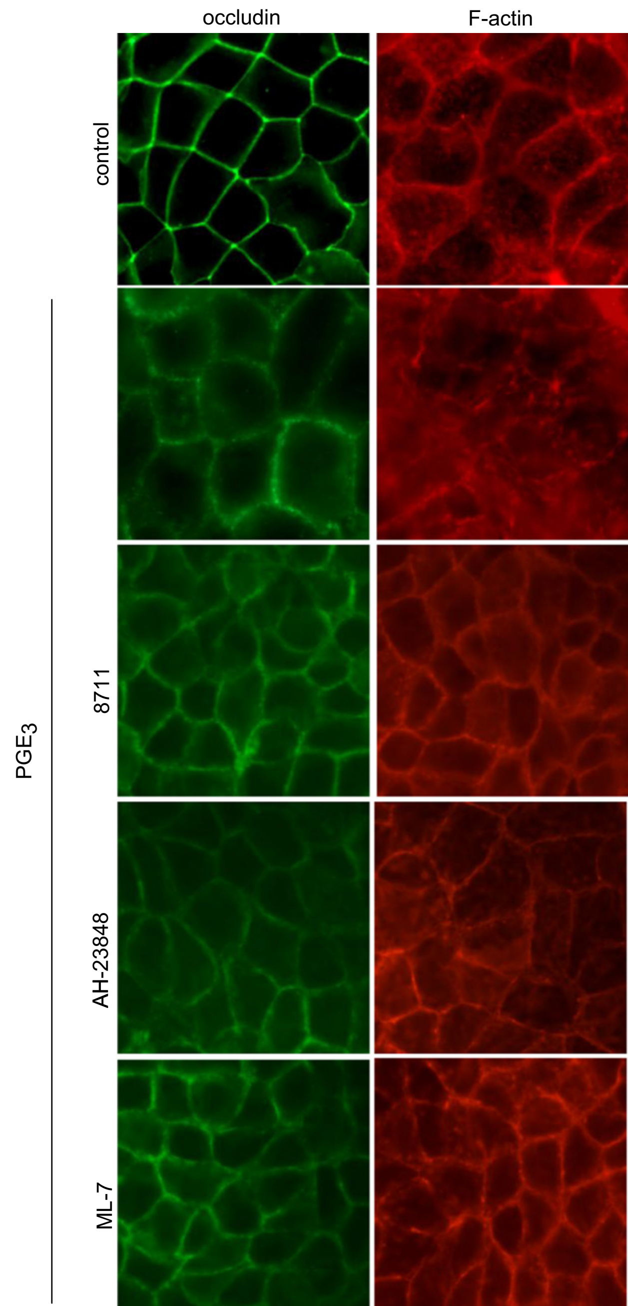


**Fig. 5.** Changes in occludin and perijunctional actin distribution induced by $PGE_3$. Cells were incubated for 3 h with $PGE_3$ (3 nM) and $PGE_3$ plus ONO-8711 (250 nM), plus AH-23848 (200 nM) or plus ML7 (0.05 μM). Fluorescent analysis was performed in cells incubated for 3 h with $PGE_3$ (3 nM) using specific occludin and ZO-1 antibodies and TRITC-phalloidin, as described in "Materials and Methods". In each case, a representative $x-y$ image of sections close to the apical cell side is shown.

a variety of gastrointestinal disorders such as IBD, irritable bowel syndrome and celiac disease [22,23]. Previous findings indicate that the AA cascade is activated in intestinal mucosa in IBD patients [24–26]. In relation to this, we previously reported that the enhancement of $PGE_2$ levels increases PP in differentiated Caco-2 cell cultures [14]. Moreover, we found that activation of $EP_1$ and $EP_4$ receptors, which activate the PLC-$IP_3$-$Ca^{2+}$ and cAMP-PKA pathways, respectively is involved in these events [13]. However, the beneficial effect of an enriched ω-3 diet on symptom alleviation in intestinal inflammatory diseases is still controversial and to date no studies of nutritional interventions in

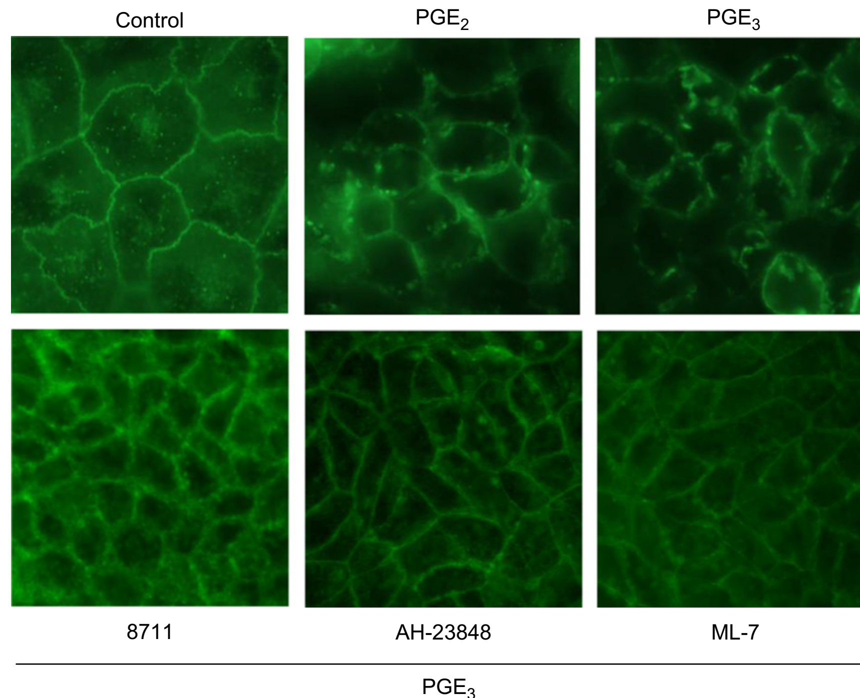


**Fig. 6.** Changes in claudin-4 distribution induced by $PGE_2$ and $PGE_3$. Fluorescent analysis was performed in cells incubated for 3 h with the PGs (3 nM) and $PGE_3$ plus ONO-8711 (250 nM), plus AH-23848 (200 nM) or plus ML7 (0.05 μM) using specific antibodies as described in "Materials and Methods". In each case, a representative $x-y$ image of sections close to the apical cell side is shown.

humans have been conclusive [5]. Taking into account all these data, the aim of this study was to assess the role of EPA-derived $PGE_3$ in the regulation of intestinal epithelial barrier function. Considering that EP receptors localization is mainly on the basolateral membrane [13], cells cultured on filters or clusters were incubated with different PGs concentrations.

Bagga et al. [27] compared the effects of $PGE_2$ and $PGE_3$ on COX-2 gene and protein expression in fibroblasts and found that both induced an increase in COX-2 mRNA. The present study demonstrates the activation of PLC-$IP_3$-$Ca^{2+}$ and cAMP-PKA pathways, through $EP_1$ and $EP_4$ interaction, respectively, as we previously reported for $PGE_2$ [13].

Willemsen et al. [28] found that the addition of EPA or DHA to intestinal cell line monolayers (T84) resulted in enhanced basal barrier integrity (TER) and in the reversion of IL-4 mediated increased permeability (FD4 fluxes). Nevertheless, there are several studies of ω-3 PUFAs such as DHA [29,30] or EPA [30,31] where they are described as being able to disrupt the epithelial barrier function of the Caco-2 cell monolayer, an effect mediated by PG formation since indomethacin, a COX inhibitor, reverted the increase in PP [30–32], thus indirectly indicating the participation of $PGE_3$ in this action. This would therefore be consistent with the results shown here, which demonstrate for the first time that $PGE_3$ is also able to induce the disruption of the epithelial barrier to an extent that is similar to $PGE_2$. Thus, EPA would have a role in PP disruption due to the action of the COX pathway metabolites. Nevertheless, we must consider that other eicosanoids such as LTs may be involved in the beneficial effects of EPA on inflammatory processes since we previously observed that EPA-derived $LTB_5$ does not have proinflammatory effects as seen with $LTB_4$ [33].

PUFA are postulated to modify intracellular signaling and several reports have been published indicating their ability to activate PKC [34,35]. Our results show that the PKC inhibitor Gö6983 was able to revert the effects induced by $PGE_3$, indicating the participation of this kinase in the downstream regulation of the TJ function.

NFκB is activated as a result of a signaling cascade triggered by extracellular inflammatory stimuli such as INF-γ, TNF-α, and IL-1β, on epithelial barrier disruption in intestinal Caco-2 cell cultures [36]. Some authors suggest a direct effect of ω-3 PUFAs on inflammatory gene expression via the inhibition or activation of the transcription factor, NFκB. In this regard, NFκB activation was also evaluated to assess its possible involvement in the $PGE_3$-induced PP events. However, we did not observe any alteration in IκB levels, indicating that the NFκB transcription factor is not a downstream target of either $PGE_2$ or $PGE_3$ in Caco-2 cells.

The delocalization of the TJ proteins, occludin and ZO-1, from TJs is associated with epithelial barrier dysfunction and increased PP [37]. Immunofluorescent examination of Caco-2 cell cultures treated with $PGE_3$ showed evidence of changes in the cellular distribution of occludin and actin through $EP_1$ and $EP_4$, while ZO-1 was not modified. Similarly, despite the dramatic redistribution of TJ proteins following IFN-γ exposure, it has been reported that ZO-1 is only minimally affected and that most of it remains at the TJ [38]. The formation of fluorescent clumps in Caco-2 cells has been attributed to a multifocal aggregation of cytoskeletal elements, including actin [39]. The same authors also proposed a central role for actomyosin contraction in the formation of these aggregates [40]. In this respect, the presence of cytosolic occludin is associated with protein internalization by endocytosis. In the case of barrier loss induced by TNF-α, occludin internalization has been described as an MLCK-dependent process [41]. Thus, the increase in PP induced by some cytokines, short chain fatty acids, ethanol, and extracellular $Ca^{2+}$ is mediated by an increase in MLCK activity [39,42–45]. Moreover, MLCK overexpression in Caco-2 cells induces the reorganization of perijunctional actin and thus an increase in PP [44]. In fact, MLCK expression is significantly enhanced in the mucosa of IBD patients [46]. Our results indicate that the increase in PP induced by $PGE_3$ correlates with the redistribution of TJ proteins (mainly occludin) and the contraction of the perijunctional actin ring through $EP_1$ and $EP_4$ interaction, and MLCK activation since PGE receptor antagonists

and ML-7 treatment was able to prevent the disruption of epithelial barrier function and changes in TJ structure. Recently, the pathophysiological relevance of claudins in the intestine has also been highlighted, as claudin-2 expression has been described to be elevated in colon epithelia of patients suffering from IBD. In contrast, the expression of claudin-4 was reduced and this protein was redistributed [47]. In general, the overexpression of claudin-4 localized within the TJ has been associated with an improvement in epithelial barrier function [48]. Nevertheless, Takehara et al. [49] found that claudin-4 overexpression in Caco-2 cells impairs barrier function. Our results showing the redistribution of claudin-4 and no effect on claudin-1 and claudin-2 by $PGE_2$ and $PGE_3$ are in accordance with Lejeune et al. [48], who observed a similar effect induced by $PGE_2$ produced by *Entamoeba histolytica.* Moreover, we demonstrated that these effects were reverted by $EP_1$ and $EP_4$ antagonists and MLCK inhibitor as we have above mentioned for occludin and actin.

On the basis of our findings, we can conclude that either ω-6- or ω-3-derived prostanoids $PGE_2$ and $PGE_3$ contribute to the regulation of epithelial barrier function through a similar mechanism [13]. Thus the previously described beneficial effect of EPA on IBD might not be attributed to the reduction in $PGE_2$/$PGE_3$ ratio as both PG has a deleterious effect on epithelial barrier function. Therefore, these findings may be taken into account for the future development of new nutritional interventions for IBD.

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