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ORIGINAL CONTRIBUTION

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Polyunsaturated fatty acids support epithelial barrier integrity and reduce IL-4 mediated permeability in vitro

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Abstract *Background* The intestinal mucosa functions as a barrier against harmful dietary and microbial antigens. An intact gut barrier forms a prerequisite for protection against infection and allergy. Both allergic and inflammatory mediators (e.g. IL-4, IFN-γ) are known to compromise the epithelial barrier integrity by enhancing permeability. Breast milk provides protection against infection and allergy and contains polyunsaturated fatty acids (PUFA). *Aim of the study* Although PUFA are commonly used in infant formulas their effect on intestinal barrier is still poorly understood. Therefore the effects of distinct PUFA (n-6: LA, GLA, DGLA, AA; n-3: ALA, EPA, DHA) and a fat blend with PUFA composition similar to that of the human breast milk fat fraction, on barrier integrity were investigated. *Methods* Human intestinal epithelial cells (T84) were pre-incubated with individual PUFA or a lipase treated fat blend, with or without subsequent IL-4 exposure. Barrier integrity was evaluated by measuring transepithelial resistance and permeability. Membrane phospholipid composition was determined by capillary gas chromatography. *Results* DGLA, AA, EPA, DHA and to a lesser extend GLA enhanced basal TER and strongly reduced IL-4 mediated permeability, while LA and ALA were ineffective. Furthermore, the lipase treated fat blend effectively supported barrier function. PUFA were incorporated in the membrane phospholipid fraction of T84 cells. *Conclusions* Long chain PUFA DGLA, AA, EPA and DHA were particularly effective in supporting barrier integrity by improving resistance and reducing IL-4 mediated permeability. Fat blends that release specific PUFA upon digestion in the gastrointestinal tract may support natural resistance.

Key words permeability – IL-4 – polyunsaturated fatty acids – arachidonic acid (AA) – docosahexaenoic acid (DHA)

Abbreviations FD4: 4 kDa FITC-dextran; IL-4: Interleukin-4; (LC-) PUFA: (Long chain-) polyunsaturated fatty acids; TER: Transepithelial resistance

Introduction

Intestinal epithelial cells play an integrate role in maintaining the intestinal barrier function and as such form the first line of defense against infectious agents or allergens. The intestinal barrier integrity is impaired in diseases like food allergy, asthma, and inflammatory bowel disease [4, 12]. During the first weeks of life, neonates have a poorly-developed intestinal barrier integrity and during postnatal intestinal maturation, gut closure progresses rapidly [37, 44]. Enhanced uptake of molecules in the intestinal mucosa may have a functional role in maturation of the mucosal immune system and induction of oral tolerance. On the other hand increased (or prolonged) permeability renders neonates more susceptible to infection, inflammation and (systemic) hypersensitivity. In developed countries the prevalence of childhood asthma and allergies is rising [24]. Secondary to allergic inflammation, intestinal permeability is increased in allergic patients, contributing to chronicity of the disease [15]. For example, mast cells and allergen specific Th_2 cells in the intestinal mucosa of allergic patients produce allergic cytokines like IL-4 capable of disrupting epithelial barrier function by enhancing both para- and transcellular permeability [5, 6, 14]. Breast milk contains bioactive components such as functional proteins like growth factors and gut hormones, oligosaccharides and polyunsaturated fatty acids (PUFA) [16]. In previous studies short chain fatty acids, fermentation products of prebiotic oligosaccharides, were found to improve the extrinsic barrier by enhancing epithelial mucus expression [46]. Besides their importance in immune development these components may support gut closure, thus providing protection against infectious diseases and development of allergies [9, 17, 31, 39]. Compared with infants fed breast milk, the gut closure period of neonates fed infant milk formula is prolonged [9]. Addition of long-chain (LC)-PUFA to infant milk formula is recommended, especially in preterm neonates, and is known to improve cognitive functions and vision during the first year of life [20]. Typically, the fat fraction of human breast milk (3–5 wt%) contains about 13–15 wt% PUFA [21]. The essential fatty acids linoleic (C18:2 n-6) and alpha-linolenic (C18:3 n-3) acid are precursors of LC-PUFA (20–22 carbon atoms) of both the n-6 and n-3 series, respectively. PUFA are incorporated into cellular membrane phospholipids, contribute to membrane fluidity and serve as precursor for eicosanoid synthesis [21, 40]. Moreover, PUFA are known to stimulate differentiation, support gut maturation, reduce transcellular permeability and may also improve tight junction formation [43, 47]. To assess whether there are qualitative differences between PUFA in their potency to improve natural resistance and gut health, an in vitro study on the effects of a series of n-6 and n-3 PUFA as well as a fat blend with PUFA composition similar to the fat fraction of human breast milk, was initiated. Effects on barrier integrity in the presence or absence of the allergy associated cytokine IL-4 were determined.

Materials and methods

PUFA abbreviations and chemicals

All PUFA and chemicals were derived from Sigma-Aldrich B.V. (Zwijndrecht, the Netherlands), unless otherwise stated. PUFA of the n-6 series were: LA (linoleic acid, C18:2 n-6), GLA (gamma-linolenic acid, C18:3 n-6), DGLA (di-homo-gamma-linolenic acid, C20:3 n-6, Cayman Chemicals (Ann Arbor, MI, USA), AA (arachidonic acid, C20:4 n-6). PUFA of the n-3 series were: ALA (linolenic acid, C18:2 n-3), EPA (eicosapentaenoic acid, C20:5 n-3), and DHA (docosahexaenoic acid, C22:6 n-3). Controls were: n-9 DGLA-analogue (C20:3 n-9) and saturated fatty acid PA (palmitic acid, 16:0). The fat blend was provided by Numico-Research B.V. (Friedrichsdorf, Germany) and porcine pancreas lipase from MP Biomedicals B.V. (Irvine, CA, USA). Methanol, ethanol, hexane, and iso-octane were from Merck (Darmstadt, Germany). Lipid standard C19:0 from Avanti Polar Lipids (Birmingham, UK) and GC reference compounds from Nu-Chek-Prep (Brunswich Chemie, Amsterdam, The Netherlands). IL-4 from Endogen (Perbio Science, Etten-Leur, The Netherlands), fetal bovine serum was from Greiner Bio-One Inc. (Longwood, FL, USA) and DMEM/F12 glutamax I, penicillin, streptomycin from Invitrogen, Breda, the Netherlands.

Preparation of PUFA dilutions

PUFA were diluted in 100% ethanol to a stock concentration of 400 mM, put in rubber cap vials and stored under nitrogen atmosphere at −80°C (methods adapted from Dooper et al. [13]). Before adding to the culture medium, the PUFA solutions were resuspended in pre-warmed fetal bovine serum enriched with ascorbic acid and alpha-tocopherol before adding to the medium. Final PUFA concentrations in the culture medium were 10 and 100 μM, with ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E)

concentrations of 75 and 20 μM, respectively (also present in control incubations without PUFA).

Fat blend and lipase treatment

The fat blend consisted of 50 w% palm oil, 20 w% coconut oil, 12 w% canola oil, 10 w% sunflower oil, 2 w% evening primrose oil, 0.6 w% fish oil, 1 w% arachidonic oil and 4 w% egg lipids; 15 w% of the fat blend consisted of PUFA (n-6: LA 12.4 w%, GLA 0.2 w%, AA 0.45 w%; n-3: ALA 1.51 w%, EPA 0.06 w%, DHA 0.30 w%). The composition of PUFA in the fat blend is similar to that of PUFA in human breast milk [20, 21]. Cells were incubated with different concentrations of the fat blend, i.e. 10–50–100 μM (calculation based on PUFA contents) together with porcine pancreas lipase (100 mU/ml) to mimic the availability of PUFA after in vivo digestion. Control incubations included fat blend without lipase treatment.

Cell culture and studies with PUFA, fat blend and IL-4

T84 human intestinal epithelial cells are commonly used to study intestinal barrier integrity in vitro. T84 cells (ATCC, Manassas, VA, USA), passage 57–64, were cultured on 12-mm transwell inserts (0.4 μm, Corning Costar, Rochester, NY, USA) in DMEM/F12 glutamax I with penicillin (100 IU/ml), streptomycin (100 μg/ml), supplemented with 5% heat inactivated fetal bovine serum. T84 cells were used 14 days after reaching confluence. Monolayers of T84 cultured on transwell filters were pre-incubated with PUFA or palmitic acid (10 and 100 μM) for 48 h and for an additional 48 h in the presence or absence of IL-4 (2 ng/ml). IL-4 was added to the basolateral compartment; medium and additives were changed every 24 h. PUFA membrane incorporation was evaluated in T84 cells grown in 6-well culture plates (Falcon, Becton Dickinson, Franklin Lakes, NJ, USA) and 10–100 μM PUFA or lipase-treated fat blend was added to the cells for 48 h (refreshed after 24 h). Cells were washed twice with PBS, scraped in 1 ml PBS, collected in glass vials and stored at −20°C.

Measurement of resistance

Epithelial barrier integrity was assessed by measuring transepithelial resistance (TER; $\Omega \cdot cm^2$) with the epithelial volt-ohm meter (EVOM; World Precision Instruments, Berlin, Germany). TER measurements were performed prior to medium refreshment at 0, 48, 72, 96 h of incubation in the presence or absence of IL-4.

Macromolecular permeability

Permeability was determined by measuring macromolecular transport of 4 kDa FITC-dextran (FD4) over the T84 monolayers after 48 h of IL-4 incubation [38]; at this time point the cells had been exposed to the PUFA or fat blend for 96 h. One hour before the determination of dextran fluxes the culture medium was replaced by medium without phenol red, and after 1h 5 μl (stock 100 mg/ml) 4 kDa FITC-dextran was added to the luminal compartment. After another hour, 100 μl sample was collected from the serosal compartment and fluorescence measured at excitation wave length 485 nm and emission 520 nm (FLUOstar Galaxy®, BMG Labtechnologies, Durham, NC, USA). FITC-dextran fluxes were calculated as pmol FITC-dextran/cm^2 per hour.

Fatty acid analyses of T84 phospholipid fraction

Fatty acid extraction of the collected T84 cells was performed according to the methods described by Blight and Dyer [7], using C19:0 as internal standard. In short, lipids were extracted from 1 ml epithelial membrane suspension under nitrogen atmosphere, by adding 2 ml methanol, 0.9 ml EDTA solution (1 g/100 ml MilliQ water) and 1 ml dichloromethane. Solid phase extraction (SPE) was used to separate the phospholipids from the other lipids in the extract. After this separation, the phospholipids were methylated using 14% BF_3 in methanol for 1 h, as described by Morrison and Smith [28]. After hexane extraction, the fatty acid methyl esters were dissolved in iso-octane and quantified by gas chromatography with a capillary column (50 m × 0.25 mm, CP-SIL88-fame). The area under the peak was automatically integrated (sensitivity 500 μg/l).

Data analyses

All data are presented as mean ± SEM. Data were analyzed with the univariate ANOVA or paired *T* test using SPSS Version 10 software.

Results

PUFA enhance basal barrier integrity of T84 monolayers and ameliorate IL-4 induced barrier disruption

Two weeks after reaching confluence T84 cells had formed monolayers with constitutive high resistance

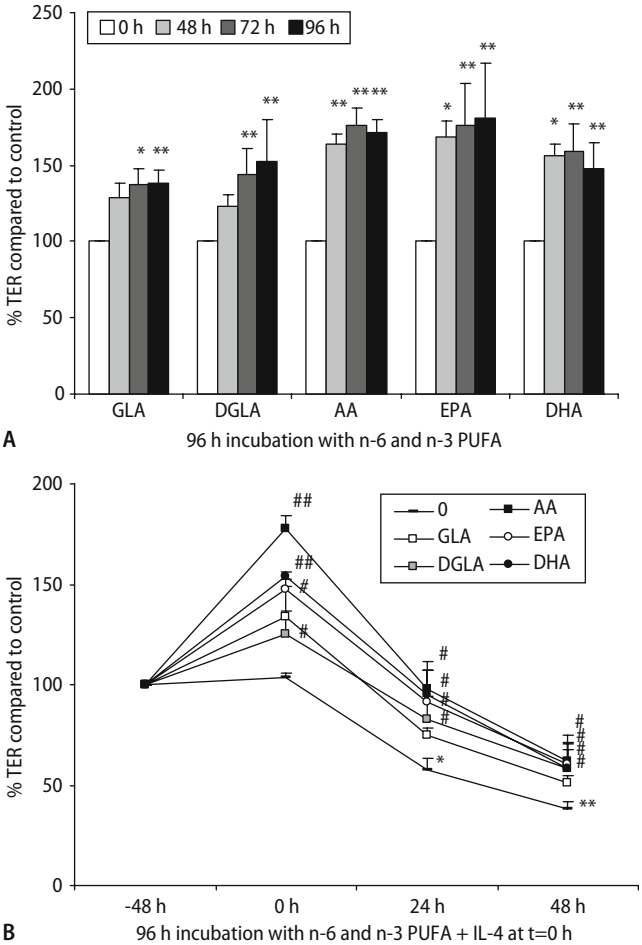

Fig. 1 PUFA enhance epithelial barrier integrity in a time-dependent manner and ameliorate IL-4 induced barrier disruption. **a** n-6 PUFA GLA, DGLA and AA and n-3 PUFA EPA, and DHA increase basal TER up to 80% after 48–96 h of incubation (100 μM, $^{*}P < 0.01$, $^{**}P < 0.005$, $n = 3$). **b** IL-4 reduces TER ($^{*}P < 0.05$, $^{**}P < 0.005$), at all time points but during co-incubation with n-6 PUFA DGLA, AA and n-3 PUFA EPA and DHA remained enhanced when compared to IL-4 control only, where GLA showed a similar tendency (**b** $\#P < 0.05$ and $\#\#P < 0.01$, $n = 3$). TER is presented as relative value (% TER). Controls are assigned 100% for each time point (not shown)

of 1,056 ± 84 $\Omega \cdot cm^2$. Incubation of these monolayers with n-6 and n-3 PUFA resulted in significant dose- and time-dependent increase in barrier resistance. Since basal resistance slightly differed between independent wells and during the course of experiments, to adequately describe kinetics, data are presented relative to controls. N-6 PUFA GLA, DGLA and AA and n-3 PUFA EPA and DHA enhanced basal resistance up to 80% at the dose of 100 μM (Fig. 1a; 96 h incubations $P < 0.005$, $n = 3$). The 10 μM dose was not effective (data not shown). GLA and DGLA significantly enhanced TER after 72 h of incubation ($P < 0.01$), while AA, EPA and DHA were already effective at 48 h of incubation ($P < 0.01$). Saturated fatty acid palmitic acid (C16:0) was ineffective (data not shown). The various PUFA did not alter basal permeability as measured by FD4 flux (data not shown). IL-4 incubations resulted in barrier disruption, with dramatic reduction of TER at 24 h of incubation ($P < 0.05$) and was further decreased after 48 h ($P < 0.005$, 38 ± 4%) (Fig. 1b, $n = 3$). To evaluate the effect of PUFA on IL-4 mediated barrier disruption cells were pre-incubated with PUFA (100 μM) for 48 h before incubation with IL-4, to allow incorporation of PUFA in the cellular membranes. After 48 h pre-incubation, the n-6 PUFA DGLA, AA and n-3 PUFA EPA and DHA all enhanced resistance and this effect was sustained during IL-4 exposure at all time points. Furthermore, GLA showed the same tendency ($P < 0.01$ and $P < 0.005$, $n = 3$).

PUFA reduce IL-4 mediated permeability

After 48 h of exposure to IL-4, the permeability for 4 kD FITC-dextran was determined and the flux calculated (Fig. 2; $P < 0.001$; control vs. IL-4, 0 ± 0 vs. 514 ± 109 pmol/cm^2 per hour; $n = 3$). In addition to the previously used PUFA, n-6 LA and n-3 ALA were tested to get a broader insight in the effectiveness of PUFA. N-6 LC-PUFA DGLA and AA and n-3 LC-PUFA EPA and DHA all were found to cause profound reduction of IL-4 mediated permeability ($P < 0.001$, $n = 3$), GLA was less effective and LA and ALA did not inhibit the FD4 flux.

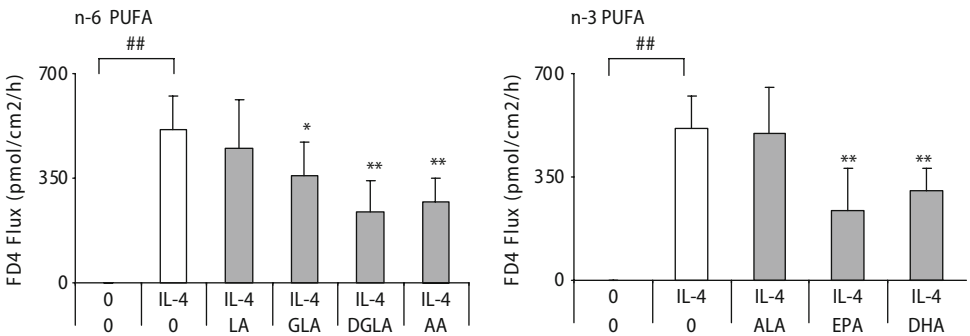

Fig. 2 T84 incubations with PUFA reduces IL-4 mediated permeability. Incubation of T84 for 48 h with allergic mediator IL-4 (2 ng/ml) resulted in pronounced increase in FD4 flux ($\#\#P < 0.001$, $n = 3$). In parallel with the effects on TER, n-6 and n-3 PUFA reduced the IL-4 mediated increased permeability. LC-PUFA DGLA, AA, EPA, and DHA were particularly effective ($^{*}P < 0.005$, $^{**}P < 0.001$, $n = 3$), whereas GLA, LA and ALA were less or not effective.

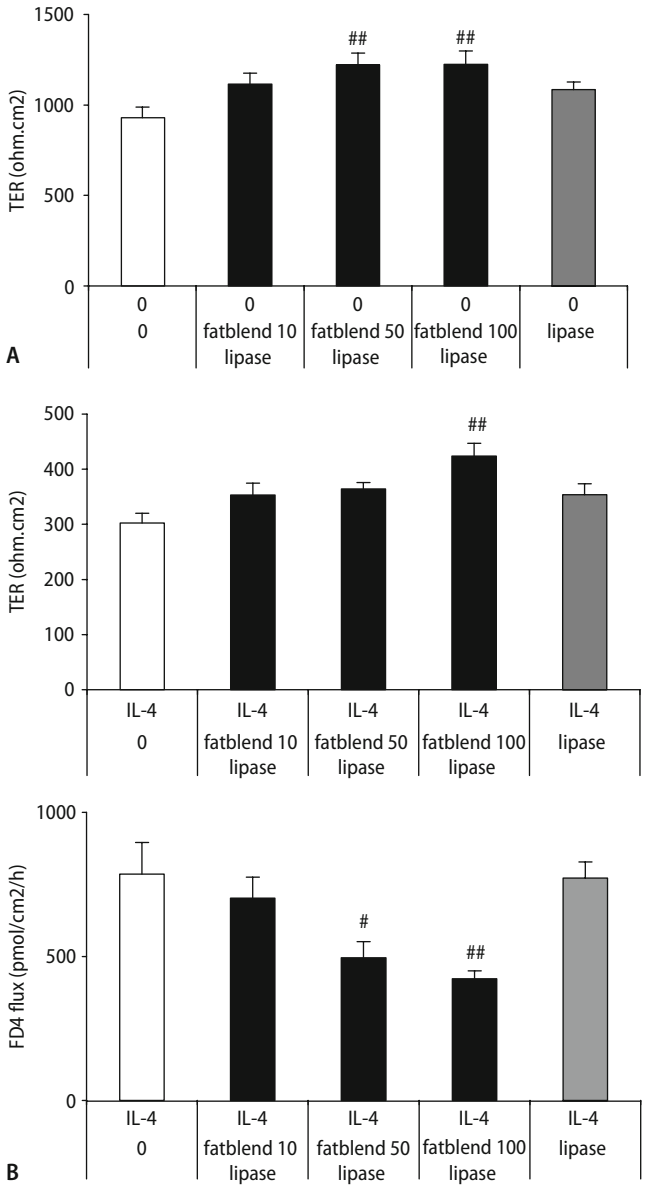

Fig. 3 Lipase treated fat blend enhances barrier integrity. **a** Fat blend (10, 50, 100 μM PUFA contents) supplemented together with lipase (100 mU/ml), enhanced basal TER in a concentration-dependent matter (compared to lipase control, ##$P < 0.001$, $n = 5$). Fat blend incubations without lipase were ineffective (data not shown). **b** Incubation with fat blend in presence of lipase, ameliorated barrier disruption as reflected by IL-4 mediated decrease in TER and increase in FD4 flux (as compared to lipase control, #$P < 0.005$, ##$P < 0.001$, $n = 5$). Fat blend and lipase were 48 h pre-incubated prior to 48 h co-incubation with IL-4

Lipase treated fat blend improves barrier integrity

Porcine pancreatic lipase (100 mU/ml) was used to simulate in vivo digestion and release fatty acids from the glycerol backbone. Similar to free PUFA, lipase-treated fat blend increased basal TER in T84 cells in a dose-dependent manner (Fig. 3a, $P < 0.001$, when compared to lipase control, $n = 5$). Lipase-treated fat blend showed a similar time dependent effect on TER compared to incubations with free PUFA. Basal FD4 fluxes were not altered during incubations with lipase-treated fat blend (data not shown). Fat blend incubations without lipase did not affect TER (data not shown). In parallel to effects on basal TER, lipase-treated fat blend, at a concentration calculated to contain 100 μM PUFA, ameliorated IL-4 mediated decrease in TER and increase in permeability compared to the lipase control (Fig. 3b, $P < 0.001$, $n = 5$). Also the 50 μM PUFA containing lipase treated fat blend was effective in reducing IL-4 mediated permeability ($P < 0.005$).

PUFA alter the composition of T84 membrane phospholipids

To assess whether the effects of PUFA on barrier integrity corresponded with incorporation in T84 cells the membrane phospholipid fraction of these cells was analyzed. The basal composition of T84 membrane phospholipids contained 0–1.5% of several n-6 and n-3 PUFA (Table 1). Forty-eight hour incubation with 100 μM PUFA resulted in incorporation of all PUFA into the cellular membrane. N-6 PUFA supplementation enriched the membrane composition for GLA, GLA, AA and LA ($P < 0.001$ for all). GLA supplementation also enhanced DGLA levels ($P < 0.005$), suggesting some elongation activity to occur in T84 cells. N-6 PUFA supplementation did not affect C16-0, C18-0 and n-3 PUFA levels. N-7 MUFA C16:1 membrane levels were diminished for about 2.5% while n-9 MUFA C18:1 was diminished for about 5% ($P < 0.001$). N-3 PUFA supplementation enriched the membrane composition for DHA, ALA and EPA ($P < 0.001$ for all). N-7 MUFA C16:1 membrane levels were diminished to 1.3% for DHA ($P < 0.05$) and up to more than 3% for EPA ($P < 0.001$), while n-9 MUFA C18:1 was diminished for about 2.5% for DHA up to 9% for EPA ($P < 0.01$). N-3 PUFA supplementation did not affect C16-0 and C18-0.

Lipase digestion resulted in a dose-dependent release of free fatty acids into the culture medium; in particular LA, GLA, AA, EPA, and DHA (data not shown). Forty-eight hour incubation with fat blend (containing 100 μM PUFA) increased incorporation of PUFA GLA, AA, EPA, and DHA only when lipase was added for digestion (Fig. 4, $P < 0.001$, $n = 5$). In addition, LA incorporation increased from 0.23 ± 0.05 to 6.87 ± 0.05% ($P < 0.001$, data not shown). Similar to incubation with free PUFA, C16:1 n-7 and C18:1 n-9 membrane levels were reduced (from 17.03 ± 0.68 to 14.33 ± 0.44 and 45.37 ± 0.67

Table 1 N-6 and n-3 PUFA are incorporated into the cellular membrane

	0	LA	GLA	DGLA	AA	ALA	EPA	DHA
C18:2 n6 (LA)	0.74 ± 0.04	9.42 ± 0.22**	0.76 ± 0.08	0.78 ± 0.03	0.83 ± 0.11	0.90 ± 0.13	0.56 ± 0.04	0.92 ± 0.21
C18:3 n6 (GLA)	0.00 ± 0.00	0.00 ± 0.00	6.63 ± 0.54**	0.21 ± 0.03	0.06 ± 0.06	0.07 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
C20:3 n6 (DGLA)	0.43 ± 0.02	0.42 ± 0.06	0.94 ± 0.10	7.22 ± 0.42**	0.42 ± 0.01	0.39 ± 0.02	0.33 ± 0.03	0.37 ± 0.02
C20:4 n6 (AA)	1.34 ± 0.06	1.23 ± 0.04	1.48 ± 0.04	1.35 ± 0.06	9.36 ± 1.40**	1.23 ± 0.05	1.29 ± 0.03	1.21 ± 0.03
C18:3 n3 (ALA)	0.00 ± 0.00	0.27 ± 0.14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	9.64 ± 1.09**	0.00 ± 0.00	0.07 ± 0.07
C20:5 n3 (EPA)	0.30 ± 0.01	0.41 ± 0.,07	0.34 ± 0.06	0.31 ± 0.01	0.47 ± 0.09	0.39 ± 0.10	14.53 ± 1.57**	0.49 ± 0.07
C22:6 n3 (DHA)	1.13 ± 0.02	1.04 ± 0.03	1.14 ± 0.06	1.10 ± 0.05	1.06 ± 0.04	1.07 ± 0.04	0.98 ± 0.04	6.09 ± 1.12**

Forty-eight hour supplementation with n-6 and n-3 PUFA (100 μM) results in incorporation of individual PUFA reaching levels between 6.1 and 14.5% of the total membrane phospholipid pool (**$P < 0.001$, $n = 3$)

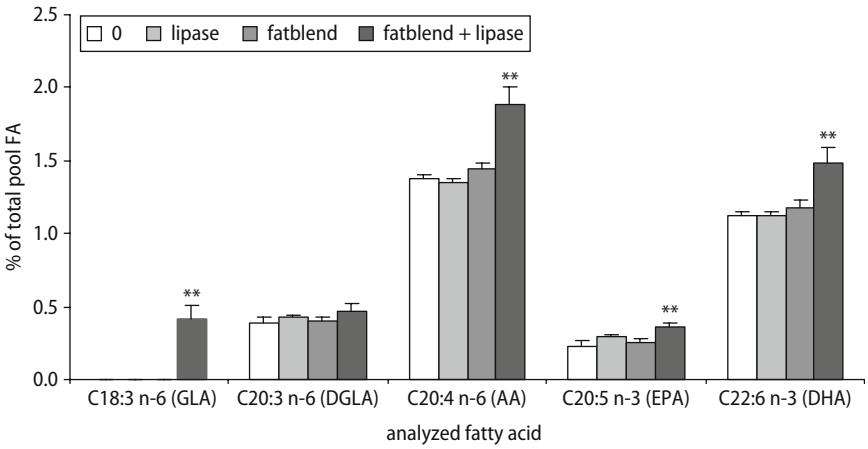

Fig. 4 Lipase treatment of a fatblend resulted in incorporation of released PUFA into the epithelial cell membrane phospholipid fraction. Incubations with 100 μM fat blend alone did not change the membrane phospholipid composition. In contrast, lipase treatment of fat blend enhanced incorporation of n-6 PUFA GLA and AA, and n-3 PUFA EPA, and DHA to 0.42–1.88% of the total membrane phospholipid pool (**$P < 0.001$, $n = 5$)

to 41.33 ± 1.91, respectively, $P < 0.001$). Only FA profiles of relevant PUFA are shown; other fatty acids were present at low levels and/or unaltered by PUFA supplementation.

Discussion

Breast milk promotes gastrointestinal and immune maturation, reduces incidence and severity of infections and reduces development of allergies [17, 31]. An intact gut barrier is a prerequisite for a strengthened immune system with regard to infection and allergy. PUFA, present in breast milk, are known to modulate the immune system and to affect the gastrointestinal development. Indeed, diets containing n-6 or n-3 PUFA reduced the trans- and paracellular permeability in the rat intestine [10]. AA and DHA have been shown to reduce the development of necrotizing enterocolitis (NEC) in neonatal rats, possibly by improving barrier function [8]. Furthermore, deficiencies in dietary PUFA were associated with increased bacterial translocation in rats, while LC-PUFA supplementation completely recovered small intestinal damage in malnourished piglets [3, 25]. Recently, in a rat model of experimental colitis n-3 PUFA were found to improve the histological scores while protecting expression and preventing redistribution of tight junction proteins [23].

In the present study both the n-6 (GLA, DGLA, AA) as well as the n-3 (EPA, DHA) PUFA enhanced basal barrier integrity (TER) in a concentration and time-dependent fashion thereby reducing IL-4 mediated permeability. In contrast, LA (n-6) and ALA (n-3) were not effective despite incorporation of these PUFA into the membrane phospholipids. Also the saturated palmitic acid and the n-9 DGLA-analogue (data not shown) were not effective.

In allergic disease the intestinal barrier function is known to be compromised as consequence of the allergic inflammation [15]. Levels of IL-4 are enhanced in the peripheral blood and/or intestinal mucosa of children affected with allergic disease such as food allergy [14]. IL-4 is derived from Th_2 and mast cells and involved in generation of the allergic effector response. The barrier disruptive effects of IL-4 on T84 monolayers have been well described, hence we therefore chose this model to test the effects of PUFA [11, 38]. In accordance with these studies, IL-4 caused a more than 60% reduction in TER and increased permeability for dextran. TER measurements determine tight junction integrity, however functional

permeability is the most sensitive marker to reveal barrier integrity when the resistance is low, e.g., after IL-4 exposure. Sanders et al. [38] have described an exponential relation between TER and flux, suggesting that at low resistance, minor enhancement in TER may cause major improvement in functional permeability. In this study LC-PUFA DGLA, AA, EPA, and DHA were found to be superior to LA, GLA and ALA in stimulating basal resistance and ameliorating IL-4 induced permeability.

In a study using Caco-2 intestinal epithelial cells, EPA and LA did not affect transepithelial resistance, however the incubation time may have been too short, i.e., 24 h [36, 42]. Roche et al. [34] found LA to slightly enhance TER during prolonged incubation with polarizing Caco-2 cells. GLA and EPA (but not LA) were found to improve barrier integrity in endothelial cells, which was associated with increased expression of tight junction protein occludin [19, 47]. Similarly, brain occludin protein levels were increased in rats fed an EPA-enriched diet [32].

Tight junction complexes actively control paracellular permeability and are sensitive targets for soluble mediators to cause barrier disruption [30, 45]. In addition to occludin, the tight junction proteins ZO-1 and claudins are important in the regulation of intestinal barrier function, which in addition is controlled by the peri-junctional-actomyosin ring and myosin light chain kinase [27]. Recently, Li et al. [22] found that EPA and DHA changed the lipid environment in tight junction membrane microdomains, preventing the redistribution of occludin and ZO-1 and reduction of resistance caused IFN-γ and TNF-α. No effects on basal barrier properties were found however short incubation time and low PUFA concentrations were used when compared to our studies. Building on the studies by Li et al. it is conceivable that in our model PUFA supported the function of the tight junction complex thereby improving transepithelial resistance. The exact mechanism by which PUFA are able to exert this effect deserves further study.

Although PUFA were able to enhance basal barrier properties, the kinetics of IL-4 mediated barrier disruption was similar in presence and absence of PUFA. This indicates that PUFA did not directly interfere with the mechanism of IL-4 mediated barrier disruption like Li et al. have found for IFN-γ and TNF-α incubations. In addition to IL-4 also IL-13 is a known allergic effector cytokine causing barrier disruption, however both IL-13 and IL-4 signal through the IL-4 receptor on intestinal epithelial cells suggesting similar mechanisms of action. In addition to IL-4, mast cell derived tryptase causes barrier disruption in allergic disease by activating the protease activated receptor-2 on intestinal epithelial cells [18]. Furthermore, antigen specific transcellular transport of the allergen–IgE complex via the epithelial CD23 receptor contributes to enhanced mucosal exposure to allergens, however this is not controlled at the level of tight junctions [48]. Future studies should reveal whether PUFA have the potential to affect these mechanisms.

In contrast to the reported protective effects of PUFA, other studies showed PUFA to diminish barrier integrity in vitro. Usami et al. and Roig-Perez et al. found that 24 h of incubation with PUFA (100–200 μM) reduced TER of Caco-2 cells, increased permeability and reduced cell viability [32, 35, 41, 42]. In the present study, no viability changes in T84 cells were observed during incubation with 100 μM PUFA for up to 96 h (WST, data not shown) possibly because vitamin C and E were added to the culture medium to avoid PUFA oxidation. Indeed, Roig-Perez et al. [35] have shown DHA to cause oxidative stress due to lipid peroxidation. Moreover, vitamin E protected Caco-2 cells from lipid peroxidation-induced cytotoxicity during incubation with PUFA [26, 29].

Like individual PUFA, the fat blend with PUFA composition similar to the human milk fat fraction, enhanced barrier integrity of T84 monolayers, the latter depending on free fatty acid release by lipase treatment. Of all human milk lipids 98% are present in the form of triacylglycerols, a glycerol backbone esterified with three fatty acids [20, 21]. In the gastrointestinal tract triacylglycerols are digested by gastric lipase, pancreatic lipase and bile salt dependent lipase [20]. In the current study pancreatic lipase was used to simulate in vivo digestion and fatty acid release. Indeed, free fatty acids were released in the culture medium (data not shown), allowing LA, GLA, AA, EPA and DHA to be incorporated in the membrane phospholipids. Incubation of T84 cells with the lipase treated fat blend had similar effects in enhancing barrier integrity as compared with individual PUFA incubations. Although the levels of LC-PUFA incorporation into the membrane phospholipids were relatively low after fat blend incubation, together with LA the additional PUFA incorporation was approximately 8%. Hence, the mixture of fatty acids available in the fat blend may have enhanced the potency of the individual fatty acids to improve barrier properties. In general, incorporation of PUFA was enhanced at the expense of C16:1 n-7 and C18:1 n-9 levels in the plasma membrane. Increased incorporation with LA, ALA and AA as well as reduction of oleic acid (C18:1 n-9) and C16:1 n-7 membrane levels are associated with increased differentiation of enterocytes [1, 2, 33]. Hence, part of the observed effect may be due to improved T84 differentiation.

This study suggests that specific PUFA of the n-6 (GLA, DGLA, AA) and n-3 series (EPA, DHA) may

support natural resistance by enhancing intestinal epithelial barrier integrity in vitro. PUFA ameliorated paracellular permeability after epithelial challenge with allergy associated cytokine IL-4. LC-PUFA, in particular, were found to enhance barrier properties, since ALA, LA and GLA were not or less effective. Our data support a beneficial role for LC-PUFA in improving gut integrity thereby contributing to the protection against infections and allergic disease.

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