A novel mechanism for gut barrier dysfunction by dietary fat: epithelial disruption by hydrophobic bile acids

Lotta K. Stenman,1 Reetta Holma,1 Ariane Eggert,2 and Riitta Korpela1
1Institute of Biomedicine, Pharmacology, Medical Nutrition Physiology, University of Helsinki, Helsinki, Finland; and 2Institute of Water and Wetland Research, Animal Physiology, Radboud University Nijmegen, Nijmegen, The Netherlands

Submitted 26 June 2012; accepted in final form 28 November 2012

Stenman LK, Holma R, Eggert A, Korpela R. A novel mechanism for gut barrier dysfunction by dietary fat: epithelial disruption by hydrophobic bile acids. Am J Physiol Gastrointest Liver Physiol 304: G227–G234, 2013. First published November 29, 2012; doi:10.1152/ajpgi.00267.2012.—Impairment of gut barrier is associated with a fat-rich diet, but mechanisms are unknown. We have earlier shown that dietary fat modifies fecal bile acids in mice, decreasing the proportion of ursodeoxycholic acid (UDCA) vs. deoxycholic acid (DCA). To clarify the potential role of bile acids in fat-induced barrier dysfunction, we here investigated how physiological concentrations of DCA and UDCA affect barrier function in mouse intestinal tissue. Bile acid experiments were conducted in vitro in Ussing chambers using 4- and 20-kDa FITC-labeled dextrans. Epithelial integrity and inflammation were assessed by histology and Western blot analysis for cyclooxygenase-2. LPS was studied in DCA-induced barrier dysfunction. Finally, we investigated in a 10-wk in vivo feeding trial in mice the barrier-disrupting effect of a diet containing 0.1% DCA. DCA disrupted epithelial integrity dose dependently at 1–3 mM, which correspond to physiological concentrations on a high-fat diet. Low-fat diet-related concentrations of DCA had no effect. In vivo, the DCA-containing diet increased intestinal permeability 1.5-fold compared with control (P = 0.016). Hematoxyl-eosin staining showed a clear disruption of the epithelial barrier by 3 mM DCA in vitro. A short-term treatment by DCA did not increase cyclooxygenase-2 content in colon preparations. UDCA did not affect barrier function itself, but it ameliorated DCA-induced barrier disruption at a 0.6 mM concentration. LPS had no significant effect on barrier function at 0.5–4.5 μg/ml concentrations. We suggest a novel mechanism for barrier dysfunction on a high-fat diet involving the effect of hydrophobic luminal bile acids.

dehydroxycholic acid; inflammation; intestinal permeability; lipopolysaccharide; ursodeoxycholic acid

THE INTESTINAL EPITHELIAL BARRIER, which is controlled by tight-junction proteins, protects the organism from the toxicity of luminal contents, such as inflammatory bacterial lipopolysaccharides (LPS) and bile acids. Several increasingly common diseases related to the Western lifestyle, such as fatty liver, steatohepatitis, and diabetes, involve leakage of the gut epithelial barrier and increased bacterial LPS in the circulation (5, 15, 21, 23). The cause of this barrier dysfunction is still unknown.

A diet rich in fat increases bile flow from the liver to facilitate fat absorption. The primary bile acids cholic acid and chenodeoxycholic acid are transformed by intestinal microbiota to their more hydrophobic secondary forms, deoxycholic acid (DCA) and lithocholic acid, whose cytotoxicity is well known. These bile acids may increase epithelial permeability to large molecular probes (14, 16, 17). We have shown that, in addition to increased total fecal bile acid concentration, a high-fat diet in mice modifies the fecal bile acid profile (19). Fatty feed increased the fecal concentration of DCA whereas it decreased the proportion of the more hydrophilic, tertiary bile acid ursodeoxycholic acid (UDCA) (19), which is potentially cytoprotective (2, 10, 18). The decrease in the proportion of fecal UDCA was related to disruption of the intestinal barrier (19).

We have previously shown in mice that a diet high in fat deteriorates gut barrier function and increases the fecal concentration of DCA nearly tenfold (19). The present study aimed to investigate whether bile acids have a role in the onset of barrier dysfunction related to a high-fat diet. The effect of hydrophobic DCA on intestinal permeability was studied in mouse jejunal and colonic tissue preparations at concentrations that correspond to those in feces of mice on a high-fat or low-fat diet. The possible protective role of the more hydrophilic ursodeoxycholic acid was also examined.

MATERIALS AND METHODS

Animals. Intestinal tissue for in vitro experiments was dissected from 6- to 11-wk-old C57Bl/6J mice obtained from Charles River (Sulzfeld, Germany). Both male and female mice were used, and they were housed in a standard animal laboratory with a dark-light cycle of 12 h:12 h. The mice were given standard chow [CRM(E), SDS, Essex, UK] and water ad libitum and were fasted for 3 h before the experiments. The animals were euthanized with 70% CO2-30% O2 (AGA, Riihimäki, Finland) and cervical dislocation. For experiments on rat intestine, we used male Sprague-Dawley rats that had participated in other feeding experiments as control animals. The animal experiments were approved by the Laboratory Animal Centre of the University of Helsinki.

Ussing chamber experiments. Fresh segments of jejunum or colon were collected for each experiment: four segments of empty jejunum were collected from a single mouse, and four segments of distal colon were obtained from two age-matched mice, two segments from each mouse. The segments were opened along the mesenteric border in cold Ringer solution, pinned onto 0.3-cm2 sliders, and mounted into an EasyMount Ussing chamber system with a voltage-clamp apparatus (Physiologic Instruments, San Diego, CA). The chamber halves were filled with 5 ml Ringer solution (120 mM NaCl, 5 mM KCl, 25 mM NaHCO3, 1.8 mM Na2HPO4, 0.2 mM NaH4PO4, 1.25 mM CaCl2, 1 mM MgSO4, 10 mM glucose, pH 7.4) on each side. The system was waterjacketed to 37°C and carbonated by a 95% CO2-5% O2 gas flow (AGA). The system was allowed to equilibrate for 10 min and solutions were replaced with fresh Ringer. At the end of each experiment (designs 1–4 described below), the chambers were washed and permeability was tested by adding fluorescein isothiocyanate (FITC)-labeled dextran (4 kDa; TdB Cons, Uppsala, Sweden) to the luminal side to a final concentration of 2.2 mg/ml. Resistance and short-circuit current were monitored during the entire experiment. Samples were taken from the serosal side at 45 min, since a longer incubation time has, in our experience, only increased variation. Fluorescence was detected with a Wallac Victor2 1420 Multilabel...
counter (Perkin Elmer, Waltham, MA). Intestinal permeability was determined by comparing serosal fluorescence to luminal fluorescence as per mille (1/1,000) translocated dextran.

**Design 1: dose dependence experiments.** Dose dependence was tested with DCA and UDCA in both jejunal and colon. Sodium deoxycholic acid was obtained from Sigma (Sigma-Aldrich, St. Louis, MO), and UDCA was kindly provided by Leiras (Helsinki, Finland).

Six experiments were performed using four different concentrations of bile acid: 0, 0.03, 0.3, and 3 mM with both jejunal and colon samples for 20 min. Six more experiments were performed with concentrations 0, 0.3, 1, and 3 mM. Using a larger molecular weight dextran (20-kDa FITC dextran, TdB Cons), we also performed six experiments with 0, 1, and 3 mM DCA. The bile acid-induced changes in tissue resistance were calculated from all experiments including control and 1 and 3 mM bile acid. The bile acids had no effect on the pH of Ringer solutions. Furthermore, there was no detectable activity of lactate dehydrogenase, a marker of cell death, in the incubation solutions. In addition, in our small pilot experiment on Caco-2 cells, transepithelial resistance decreased 70% by 20 min of incubation with DCA but was completely restored after 24 h, indicating a reversible effect for DCA (Ventola H, unpublished data).

**Design 2: UDCA in DCA-induced barrier dysfunction.** To test the possible protective effect of UDCA in DCA-induced barrier defect, luminal DCA (3 mM) was used with four different concentrations of UDCA: 0.1, 0.3, 0.6, and 1 mM. The bile acids were coincubated with the tissue for 20 min before permeability measurements. Each experiment was performed on both mouse jejunal and mouse colon with Ringer solution as negative control, 3 mM DCA as positive control, and two concentrations of UDCA in each experiment. A total of 20 experiments were conducted.

**Design 3: dexamethasone treatment.** To investigate whether the barrier impairment seen by DCA in 20 min is inflammation dependent, we used 1 μM water soluble dexamethasone (D2915, Sigma-Aldrich) on the luminal side of mouse jejunal and colon. The drug was coincubated with 3 mM DCA for 20 min before permeability measurements.

**Design 4: LPS and intestinal permeability.** We hypothesized that LPS aggravates DCA-induced barrier dysfunction but does not increase intestinal permeability alone. To test this in mouse distal colon, we induced a barrier defect with 3 mM DCA for 20 min, washed the chambers twice with Ringer solution, and added LPS from Escherichia coli O55:B5 (L2880, Sigma-Aldrich) in concentrations of 0.5, 1.5, and 4.5 μg/ml for 60 min. To test whether LPS has an effect on intestinal permeability independently of DCA, we incubated 0.5 and 4.5 μg/ml LPS with segments preincubated with Ringer solution only.

**Tissue preparation for protein analysis.** Distal colon was dissected from male and female 12- to 13-wk-old C57Bl/6d mice (Charles River). The mice were euthanized with a gas mixture of 70% CO2-30% O2 (AGA) and cervical dislocation. The distal colon was cut into four segments, each of which was placed in one of four solutions: 1) Ringer, 2) Ringer with 3 mM DCA, 3) Ringer with 3 mM UDCA and 0.3 mM UDCA, and 4) Ringer with 3 mM DCA and 0.6 mM UDCA. Segments were incubated for 20 min at 37°C, until either snap-frozen in liquid nitrogen and stored in −80°C for Western blot analysis or fixed in 10% neutral buffered formaldehyde (Sigma-Aldrich) for 24–48 h and embedded in paraffin for immunohistochemistry or hematoxylin-eosin staining.

**Western blot analysis.** Samples were prepared in RIPA buffer, and protein content was measured with a Pierce BCA Protein Assay kit (Thermo Scientific, Waltham, MA). Diluted samples were further diluted 1:1 into Laemmli sample buffer with mercaptoethanol. SDS page was run on 7.5% gels, and proteins were blotted onto commercial PVDF membranes (Bio-Rad, Hercules, CA) by use of a TransBlot Turbo device (Bio-Rad). Membranes were blocked with 0.5% milk-Tris-buffered saline (TBS) solution with 0.05% Tween 20 solution using Snap-i.d. for all incubations (Millipore, Billerica, MA). The following dilutions were used for antibodies: rabbit β-actin 1:300 (no. 49675, Cell Signaling Technology, Danvers, MA), anti-rabbit 1:500 (no. 170–6515, Bio-Rad), goat cyclooxygenase-2 (COX-2) 1:200 (sc-1747, Santa Cruz Biotechnology, Santa Cruz, CA), and anti-goat 1:2,000 (sc-2020, Santa Cruz Biotechnology). COX-2 was blotted from seven tissue preparations per group. Bands were detected with ECL Plus substrate (RPN2132, Amersham ECL Plus Western Blotting Detection System, GE Healthcare, Fairfield, CT) and an FLA Image Reader (Fujiﬁlm, Tokyo, Japan). Bands were quantiﬁed with the inverted-peaks technique using ImageJ (open source software).

**Immunohistochemistry.** Slides were incubated in xylene (2 × 5 min), 95% ethanol (5 min), 70% ethanol (5 min), 50% ethanol (5 min), and TBS-Tween 20 (3 min). Protease treatment was conducted in 1 mg/ml Streptomyces griseus protease (Sigma-Aldrich) for 10 min at 37°C. After being washed with TBS-Tween 20 (2 × 5 min), slides were blocked with 1% bovine serum albumin (Biotop, Turkü, Finland) diluted in TBS for 2 h at room temperature. Samples were circled with a PapPen (Invitrogen). A primary antibody for occludin (mouse anti-occludin 33–1500, Invitrogen) was added at 1:100 dilution, 300 μl on each slide, and samples were incubated overnight at 6°C. After being washed with TBS (2 × 5 min), the secondary antibody (anti-mouse FITC, Fisher Scientific, Waltham, MA) was added (1:200, 300 μl on each slide) followed by incubation for 1 h at room temperature. The slides were washed (3 × 3 min in TBS), and Fluoroshield with 4′-6-diamidino-2-phenylindole (Fluoroshield with DAPI, Sigma-Aldrich) was used to stain nuclei. The slides were covered with ProLong Gold Antifade Reagent (Invitrogen) and imaged with a fluorescence microscope (Axio Imager M2, Carl Zeiss, Oberkochen, Germany). Images for nuclei and occludin were merged by use of ImageJ software.

**In vivo feeding trial of DCA.** Eleven-week-old male C57Bl/6d mice obtained from Charles River and housed in a standard animal laboratory with a 12:12-h light cycle and food and water ad libitum. Diets were made with the Lantmannen lantbruk R36 diet (Stockholm, Sweden), with 400 ml water added in 800 g of meal. The control group (n = 12) received this diet throughout the study. The DCA group received a diet containing 2 g/kg (n = 12) DCA for an initiation period of 1 wk. Since the group receiving DCA began to lose weight, the dose was halved to 1 g/kg for the remaining weeks. Half of the mice were euthanized at week 5 (n = 6+1), and half at week 10 (n = 6+6). One of the 5-wk control mice died during the experiment because of an error in handling. At the end of the experiment, mice were fasted overnight and gavaged with 500 mg/kg FITC dextran (4 kDa, TdB Cons). After 4 h mice were euthanized with a gas mixture of 70% CO2-30% O2 (AGA), and blood was collected by decapitation. Intestinal permeability was determined by diluting serum 1:5 to PBS and measuring fluorescence with a Wallac Victor2 1420 Multi-label counter (Perkin Elmer). Serum of a nongavaged mouse was used to obtain a blank value, and fluorescence values were set against a standard curve for 4-kDa FITC dextran to acquire dextran concentrations.

**Data analysis.** In in vitro studies, differences between groups were calculated by using the nonparametric Kruskal-Wallis test and PASW software version 18.0.2 (IBM, Armonk, NY). If global P < 0.05, differences between groups were analyzed via the Mann-Whitney U-test. The effect of LPS on DCA-induced barrier dysfunction was analyzed by comparing DCA+LPS groups to the DCA control by the nonparametric paired Wilcoxon test. Groups with only LPS were compared with the control incubated in Ringer solution only. In the in vivo study, a 2 × 2 factorial test (univariate analysis of variance) was used, since the study contained two fixed factors: diet (control and DCA) and duration (5 and 10 wk). This test shows the independent effect of these two factors and any possible interaction. P < 0.05 was considered statistically significant in all analyses.

**RESULTS**

**Dose dependence of DCA in gut permeability in vitro.** DCA increased intestinal permeability dose dependently in both jeju-
num and colon (Fig. 1, A and B). A significant increase in permeability was noted at concentrations of 1 mM (1.8-fold increase in median in jejunum, 1.9-fold in colon) and 3 mM (2.2-fold increase in median in jejunum, 3.5-fold in colon), whereas the concentrations 0.03 and 0.3 mM had no significant effect. DCA also increased permeability to the larger 20-kDa dextran at a 3 mM concentration in colon (Fig. 1D), but not in jejunum (Fig. 1C). Tissue resistance was decreased by 3 mM DCA in colon [0 mM: −16.2% (SE 4.46%, n = 12); 1 mM: −13.6% (SE 2.74%, n = 12); 3 mM: −29.1% (SE 3.95%, n = 12); P < 0.05 for 3 mM vs. 0 and 1 mM], but not in jejunum (data not shown).

Effect of DCA on gut permeability in vivo. A diet supplemented with 0.1% DCA impaired gut barrier function in a 10-wk feeding trial in mice (factorial test, P for diet = 0.016, P for duration = 0.27) (Fig. 2).

UDCA and gut permeability. UDCA did not affect permeability in either jejunum (global P = 0.10) or colon (global P = 0.75) (Fig. 3, A and B), although there seemed to be a tendency for decreased permeability in jejunum at 0.3 mM (P = 0.03). UDCA had no effect on tissue resistance in either jejunum [global P = 0.29; delta resistance during bile acid incubation, 0 mM: −12.0% (SE 4.8, n = 12); 1 mM: −8.5% (SE 2.8, n = 6); 3 mM: 8.3% (SE 8.3, n = 12)] or colon [global P = 0.13; 0 mM: −13.3% (SE 2.8, n = 12); 1 mM: 0.6% (SE 8.3, n = 6); 3 mM: 2.8% (SE 4.2, n = 12)]. UDCA protected colon against DCA-induced barrier dysfunction at 0.6 mM (P < 0.05) with a more than 50% decrease in the median value of permeability, and there was a decreasing trend at 0.1 mM and 0.3 mM concentrations (Fig. 3D). UDCA had no significant protective effect against DCA in jejunum (Fig. 3C). UDCA had no effect on the DCA-induced changes in tissue resistance (data not shown).

Colonic morphology and occludin content. Tissue morphology was dramatically altered by bile acid incubation. Both hematoxylin-eosin staining and immunofluorescence staining with occludin revealed that the colonic epithelium was disrupted by 3 mM DCA (Fig. 4). All images are representative of three animals. Deoxycholic acid did not seem to affect occludin content in the colon, as measured by Western blot analysis.

**Fig. 1. Effects of deoxycholic acid (DCA) on mouse intestinal permeability: to 4-kDa FITC dextran in jejunum (A) and colon (B) and to 20-kDa FITC dextran in jejunum (C) and colon (D). Experiments were conducted in Ussing chambers. Box plots show median with upper and lower quartiles. Whiskers show minimum (min) and maximum (max) with outliers (Tukey) indicated as black dots. A and B: 0, 0.3, and 3 mM; n = 12; 0.03 and 1 mM; n = 6. C and D: n = 6 per group. *P < 0.05, **P < 0.01, ***P < 0.001; NS, not significant.
from three tissue samples (data not shown). We also measured occludin content from two groups with DCA and 0.3 or 0.6 mM UDCA, and there was no difference in occludin content in any of the groups.

**Dexamethasone treatment and colonic COX-2 content.** DCA-induced barrier defect was not prevented by anti-inflammatory dexamethasone treatment either in jejunum (P = 0.44; Fig. 5A) or in colon (P = 0.52; Fig. 5B). Furthermore, COX-2 content was not increased in colon in a 20-min incubation with 3 mM DCA (P = 0.57) (Fig. 6).

**LPS and gut permeability in intact and DCA-treated colon.** LPS treatment seemed to aggravate DCA-induced barrier dysfunction by doubling permeability at a concentration of 4.5 μg/ml (P = 0.075; Fig. 7A). LPS had no effect on the permeability of intact mouse intestinal tissue.

To seek support for these data, we conducted experiments on the distal colon of Wistar rats. We used 1 and 5 μg/ml LPS together with 3 mM DCA. DCA increased colonic permeability more than twofold. Mean colonic permeability to dextran was slightly higher in both LPS groups compared with DCA only, and in a generalized estimated equations analysis 5 μg/ml LPS with DCA tended to aggravate barrier dysfunction (P = 0.05; Fig. 7B). LPS had no effect on the permeability of intact rat intestine.

**DISCUSSION**

The purpose of this study was to investigate the potential pathogenic role of bile acids in dietary fat-related barrier dysfunction by incubating mouse jejunal and colonic segments with DCA and UDCA in an Ussing chamber. We further investigated the effect of DCA on epithelial integrity and inflammation by using histological evaluation, immunofluorescence staining, and Western blot analysis. The role of inflammation was examined by studying dexamethasone and LPS in DCA-induced barrier dysfunction and by analyzing the effect of DCA on colonic COX-2 content by the Western blot technique. We here show with four different methods that DCA impairs gut barrier function, especially in the colon. This is the first study to show that DCA impairs gut barrier function in vivo.

DCA incubation resulted in a dose-dependent jejunal and colonic barrier dysfunction in vitro but did not increase jejunal permeability to the 20-kDa FITC dextran. The concentration of DCA in feces of fat-fed mice varies from 1.4 to 3.0 mM and in control mice from 0.28 to 0.44 mM (19). Interestingly, in the present study DCA did not influence colonic barrier function at the lower fecal concentrations. However, at a sixfold higher concentration, which corresponds to the fecal concentration in fat-fed mice, DCA more than doubled colonic permeability. A detrimental effect of DCA on epithelial integrity has been reported (16, 17), and we here show that a fat-diet-related concentration of DCA is strongly related to barrier dysfunction, whereas a concentration related to low-fat feeding does not impair barrier function.

DCA feeding impaired barrier function in mice in vivo. Intestinal permeability was 1.5-fold in DCA-fed mice compared with controls. In our previous study in mice on a high-fat diet with a measured fecal DCA concentration of 1.4–3 mM (19), the elevation in permeability was modest, only 15 and 30% in jejunum and colon. In the present in vivo study, a calculated estimation of the fecal concentration of DCA in the bile acid-fed mice would be 15 mM. Since this concentration is fivefold to the concentration in the feces of high-fat-fed mice, it is not surprising that the difference in effect size in the two studies is of a similar magnitude.

The present results show that UDCA protects against DCA-induced barrier dysfunction in colon, but not jejunum. Since most bile acids are absorbed in the ileum, the bile acid concentration is two- to threefold in jejunum compared with colon, as reported in rats (11). The differences in physiological concentrations of bile acids may result in different defense mechanisms in these two intestinal loci. This, in turn, may explain the different results in jejunum and colon.

We have previously reported an inverse correlation between the proportion of fecal UDCA and intestinal permeability (19). Orally administered UDCA has shown barrier-protective effects also in experimentally induced ileitis in rats (2). The present results give rise to the hypothesis that a decreased proportion of protective luminal bile acids, such as UDCA, results in the increased ability of cytotoxic bile acids like DCA to induce barrier dysfunction on a high-fat diet.

In the present study, the effective concentration of UDCA for prevention of barrier dysfunction was tenfold the physiological concentration of UDCA in mice on a high-fat diet (19). It is likely that the organism is incapable of producing the high concentration of UDCA required to reduce DCA-induced damage in the gut barrier. Hence, the increase in DCA concentration seems to be an important factor in setting bile hydrophobicity off balance.

The low concentration of UDCA in feces may also indicate the importance of other luminal factors intertwined with UDCA’s effect on barrier function. One such factor may be β-murich-
olic acid, another hydrophilic bile acid, which is produced primarily in rodents. This bile acid has been shown to have a beneficial effect on cholesterol gall stones, similar to that of UDCA (22). In a postanalysis of our mouse fecal samples, we noted a drastic decrease to one fifth in the ratio of muricholic acid to deoxycholic acid resulting from a high-fat diet (Stenman LK, Holma R, and Korpela R, unpublished data).

To further elucidate the underlying mechanisms of DCA-induced barrier dysfunction, we performed hematoxylin-eosin and immunofluorescence occludin staining for segments of colon. The stainings show considerable disruption of the colonic epithelium, which was not reflected in tissue occludin content. As a surfactant, DCA is known to disrupt lipid bilayers (8). On the contrary, the hydrophilic bile acid UDCA stabilizes lipid bilayers (10). The opposite actions of these two bile acids on lipid bilayers may explain why UDCA reduced DCA-induced modifications in tissue structure, which was also reflected in reduced tissue permeability.

To see whether DCA induces barrier dysfunction via an inflammatory mechanism, we tested whether dexamethasone could inhibit DCA-induced barrier dysfunction. Dexamethasone proved ineffective in this respect. Furthermore, the COX-2 content in colon remained unaffected. Thus our data suggest that the primary mechanism of bile acid-induced barrier dysfunction is rather the tissue-disrupting effect of DCA and not inflammatory as in vitro data have suggested (1, 14,

Fig. 3. Effect of ursodeoxycholic acid (UDCA) on jejunum (A) and colon (B) and the effect of DCA alone and simultaneously with UDCA on intestinal permeability in jejunum (C) and colon (D). Permeability was measured as translocation of 4-kDa FITC-dextran in Ussing chambers. Box plots show median with upper and lower quartiles. Whiskers show min and max with outliers (Tukey) indicated as black dots. A and B: 0, 0.3, and 3 mM: n = 12; 0.03 and 1 mM: n = 6. C and D: n = 20 for control and DCA only, n = 10 for groups with DCA + UDCA. *P < 0.05, ***P < 0.001.
17). These previous studies may not be applicable to fat-induced barrier dysfunction, since the bile acid concentrations employed were subphysiological (10–250 μM). Although acute bile acid exposure did not increase COX-2 content in the present study, we do not rule out the possibility that a long-term challenge by luminal bile acids may induce mucosal inflammation in the intestine.

We also tested the effect of LPS on gut permeability. Gut microbiota and gut-derived LPS are thought to be important mediators in the pathologies related to barrier dysfunction (6). Although small-intestinal bacterial overgrowth (with potentially increased LPS levels) is comanifested with increased gut permeability in nonalcoholic fatty liver disease (15), there is no evidence that increased intestinal LPS levels are involved in

![Fig. 4. Morphology of colonic segments after treatment with bile acids. Tissue preparations were incubated in Ringer solution (Control) or DCA or UDCA for 20 min before fixation in formaldehyde. Slides were stained with immunofluorescence (A and B) or hematoxylin-eosin (C). Immunofluorescence stainings show occludin in green and nuclei in blue. Magnifications ×20 (A and C) and ×40 (B).](image)

![Fig. 5. Effect of dexamethasone (Dex) on DCA-induced gut barrier dysfunction in jejunum (A) and colon (B). DCA was used at a 3 mM concentration. Permeability was measured as translocation of 4-kDa FITC-dextran in Ussing chambers. Box plots show median with upper and lower quartiles. Whiskers show min and max with outliers (Tukey) indicated as black dots; n = 10 per group.](image)
the onset of barrier dysfunction. The present study shows that LPS has no effect on the permeability of intact mouse colon when administered to the luminal side. These data are in concordance with reports showing that apical LPS does not increase the permeability of the colon (9) or intestinal Caco-2 cells (12) even at concentrations 100 times greater than the ones we used. The results suggest that LPS does not have a role comparable to that of DCA in the initiation of barrier dysfunction.

A trend toward LPS-aggravated barrier dysfunction could be seen, however, when the mucosal barrier was first damaged with DCA. Münch et al. (16) have reported that chenodeoxycholic acid and deoxycholic acid increase the passage of LPS into human gut mucosa. LPS increases the permeability of Caco-2 cells when administered to the basolateral side (12). In humans, systemically given LPS increases intestinal permeability (13). These reports provide a basis for the claim that LPS aggravates barrier dysfunction and is involved in the pathogenesis of disorders related to a leaky gut but is not involved in the initiation of luminally derived barrier dysfunction.

We suggest a novel hypothesis that barrier dysfunction on a high-fat diet is initiated by an altered luminal bile acid profile. As we have shown, dietary fat decreases the proportion of fecal UDCA, as the concentration of DCA is increased (19). According to the present data, these alterations may increase intestinal permeability.

Recent data have linked diet-induced obesity to barrier dysfunction (4, 7, 19, 20). It is thus far unclear whether this dysfunction is attributable to obesity or to a predisposing diet. Leptin-deficient obese mice have been reported to develop a leaky gut (3), whereas other authors have reported barrier dysfunction in diet-induced obese mice (4, 7). However, a recent rat study noted increased permeability only on a fat-rich diet, but not in genetic obesity (20), which would suggest that dietary fat is responsible for diet-derived barrier dysfunction. The authors noted an increased serum bile acid concentration and thus proposed a fat-induced increase in bile flow as an

---

Fig. 6. Effect of 3 mM DCA on colon COX-2 content in vitro. Tissue preparations were incubated in Ringer solution (Control) or DCA for 20 min before freezing and protein extraction. Cyclooxygenase-2 (COX-2) enzyme was also run on electrophoresis gels to better demonstrate the protein. Box plots show median with upper and lower quartiles. Whiskers show min and max; n = 7 per group.

Fig. 7. Effect of lipopolysaccharide (LPS) on colonic permeability alone and in barrier dysfunction induced by 3 mM DCA in mouse (A) and rat (B). Permeability was measured as translocation of 4-kDa FITC-dextran in Ussing chambers. Box plots show median with upper and lower quartiles. Whiskers show min and max with outliers (Tukey) indicated as black dots; n = 7–24 per group.
underlying mechanism for impaired barrier function (20). We have shown an altered fecal bile acid profile in barrier dysfunction induced by a fatty diet (19). The present results reveal that alterations in bile acid metabolism are related to the pathogenesis of fat-induced barrier dysfunction in mice.

In conclusion, using murine jejunal and colonic preparations in an Ussing chamber and DCA-feeding in vivo, we demonstrated that luminal bile acids have a role in the onset of barrier dysfunction related to a diet high in fat. DCA induced barrier dysfunction at concentrations related to a fat-rich diet in mice, but not at concentrations related to a control diet. The effect was ameliorated by administration of UDCA. DCA had a tissue-disrupting, rather than inflammatory, effect. LPS alone did not affect barrier function, but it aggravated barrier disruption induced by DCA. We present here data suggesting that alterations in luminal bile acid profile are a key factor in the pathogenesis of barrier dysfunction related to a fat-rich diet.

ACKNOWLEDGMENTS

We thank Ilkidiö Hyötyen and Aino Siltari for help with the Ussing chamber experiments, Tuomas Heini for help with Western blotting, Hanna Keränen for help with immunohistochemistry and mouse work, and Hanna Ventola for sharing expertise in cell cultures. Statistician Tuija Poussa is acknowledged for statistical advice. We are grateful to Professor Eero Mervaala and Professor (emeritus) Heikki Vapaatalo for critical discussions.

GRANTS

This work was supported by a grant from The Finnish Funding Agency for Technology and Innovation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

19. Suzuki T, Hara H. Dietary fat and bile juice, but not obesity, are responsible for the increase in small intestinal permeability induced through the suppression of tight junction protein expression in LETO and OLETF rats. Nutr Metab (Lond) 7: 19, 2010.