Higher Fecal Bile Acid Hydrophobicity Is Associated with Exacerbation of Dextran Sodium Sulfate Colitis in Mice\textsuperscript{1–3}

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Abstract

Increased luminal bile acid hydrophobicity is associated with cytotoxicity and has been suggested to contribute to gut barrier dysfunction. The aim of this study was to compare 2 high-fat diets and a low-fat diet as to whether they modify fecal bile acid profile and hydrophobicity and/or susceptibility to dextran sodium sulfate (DSS) colitis in C57Bl/6J mice. Control and DSS-Control groups received a low-fat control diet [5.5% of total energy (E%) soy oil, 4.5 E% lard], and the DSS-Lard (5.5 E% soy oil, 54.5 E% lard) and DSS-Fish oil (5.5 E% soy oil, 27.2 E% lard and 27.2% menhaden oil) groups received high-fat diets. Feces for bile acid analysis were collected after 3-wk feeding, followed by induction of dextran DSS colitis (2 d 5% DSS in drinking water + 2 d tap water). Fecal bile acid hydrophobicity was elevated 76% in the lard group ($P$ = 0.051) and 122% in the fish oil group ($P$ = 0.001) compared with control, indicating potentially increased cytotoxicity. DSS caused severe colitis symptoms, evaluated as rectal bleeding, whereas all the controls were symptom free. The median symptom scores were: DSS-Control, 2.3 (IQR = 0.6, 3.0), DSS-Lard, 0.3 (IQR = 0, 2.3), and DSS-Fish oil, 2.4 (IQR = 1.9, 2.8). The only differences were DSS-Control vs. control ($P$ < 0.001) and DSS-Fish oil vs. control ($P$ < 0.001). Severity of symptoms in all colitic mice was positively correlated with fecal bile acid hydrophobicity (Spearman’s $r$ = 0.43; $P$ = 0.028) and fecal deoxycholic acid concentration (Spearman’s $r$ = 0.39; $P$ = 0.048). These results suggest that luminal bile acid modification, induced by altered dietary fat composition, may alter susceptibility to DSS colitis. J. Nutr. 143: 1691–1697, 2013.

Introduction

Bile acids are produced by the liver and excreted into the duodenum as conjugated bile salts to facilitate absorption of dietary fat. Primary bile acids include cholic acid, chenodeoxycholic acid, and β-muricholic acid, the last of which is specific to rodent bile (1). In the large intestine, bile salts are deconjugated into bile acids and undergo various microbial transformations into secondary bile acids, such as deoxycholic acid and ursodeoxycholic acid (2). These secondary bile acids vary in hydrophobicity and cytotoxicity. Deoxycholic acid (DCA)\textsuperscript{7} is very hydrophobic and capable of disrupting cell membranes, causing cytotoxicity, with the potential mechanisms being: solubilization of cell membranes (3), production of reactive oxygen species (4), and epithelial growth factor receptor activation and tight-junction redistribution (5). In contrast, ursodeoxycholic acid (UDCA) is more hydrophilic and can stabilize lipid membranes to prevent disruption by deoxycholic acid (3,6).

It was recently shown that a diet high in fat [>45% of total energy (E%)] impairs gut barrier function (7–11) by unknown mechanisms of action. We have suggested that alterations in luminal bile acid profile contribute to barrier dysfunction (11). Dietary fat increases the fecal concentration and proportion of DCA (11,12), which increases intestinal permeability both in vivo, as we have shown (13), and ex vivo in several species, including humans (14–17). We have also shown that the proportion of UDCA in fecal bile acids is decreased in mice fed with a high-fat lard diet (11). In our recent study, UDCA protected colonic segments of mice from barrier dysfunction by DCA (13). These findings may indicate a role for luminal bile acids in the pathogenesis of gut barrier dysfunction associated with a diet high in saturated fat.

Dietary fat increases the fecal excretion of bile acids (12,18–20), but it is unclear whether fat quality affects total bile acid excretion. Moreover, very little is known of how dietary fat...
quality effects fecal bile acid profile. As far as we know, only 4 studies have quantified an array of bile acids from feces after feeding rats with various dietary fats (12–21,23), but none of them have calculated variables describing bile acid hydrophobicity.

Increased gut permeability is thought to increase translocation of antigens across the tight-junction barrier of the gut epithelium by a mechanism that may be paracellular or transcellular. These antigens are an important factor in the pathogenesis of inflammatory bowel diseases (24). Although dietary fat is a potential risk factor for these diseases (25), no studies have investigated the role of luminal bile acid profile in susceptibility to colitis.

The aim of this study was to investigate whether a diet with 60% lard or lard with fish oil affects susceptibility to dextran sodium sulfate (DSS) colitis via a potential mechanism related to luminal bile acid profile and hydrophobicity.

**Materials and Methods**

**Mice and diets.** Male C57Bl/6J mice (n = 40) were obtained from Charles River and housed in standard laboratory conditions with a 12-h light-dark cycle and free access to food and water. At 8–9 wk of age, mice were divided into the following 4 groups to undergo a dietary intervention of 4 wk: Control (low-fat diet and no DSS treatment), DSS-Control (control diet and colitis induced with DSS), DSS-Lard (high-fat lard diet and colitis induced with DSS), and DSS-Fish oil (high-fat diet with lard and fish oil and colitis induced with DSS). The control groups (Control and DSS-Control) received a standard low-fat diet containing 10% fat comprised of 5.5% soy oil and 45% E% lard (D12450B, Research Diets). The high-fat groups received a diet with 60% E% fat. The lard diet was comprised of 5.5% soy oil and 45.5% E% lard (D12492, Research Diets), whereas the fish oil diet contained 10% fat comprised of 5.5% soy oil, 27.2% E% lard, and 27.2% menhaden oil (D9020506, Research Diets). The fish oil diet was ordered in air-tight, zip-locked bags and stored in a freezer at −20°C. Fresh pellets were made available 4–6 times/wk. The contents of the diets are shown in Supplement Table 1. The DSS-Control, DSS-Lard, and DSS-Fish oil groups were exposed to DSS after dietary intervention. Animal experiments were approved by the Animal Experiment Board in Finland.

**Analysis of fecal bile acids.** At week 3 of the study, before colitis induction, mice were housed 4 h in a metabolic cage to collect fecal samples. Samples were dried overnight in a nitrogen gas flow and pulverized. Bile acids were extracted and analyzed by GLC as previously described (26). 5α-Cholesterol was used as an internal standard. The following bile acids were standardized: cholic acid, chenodeoxycholic acid, β-muricholic acid, deoxycholic acid, lithocholic acid, ursodeoxycholic acid, lithocholic acid, and epideoxycholic acid. A fecal hydrophobicity index was calculated as a percentage-weighted mean of previously reported hydrophobicitles (27) for 6 bile acids, cholic acid, chenodeoxycholic acid, β-muricholic acid, deoxycholic acid, lithocholic acid, ursodeoxycholic acid, and epideoxycholic acid, using estimated values for lithocholic acid (1.13) and β-muricholic acid (~0.65).

**Induction of DSS colitis.** At the beginning of week 4, mice ad libitum consumed a 5% solution of DSS as their only drinking water for 2 d (36–50 kDa, MP Biochemicals) while all mice continued to receive their designated diets until the end of the experiment. There were no major differences between groups in daily drinking behavior (mean ± SEM, g/mouse) during the 2-d DSS administration (4.0 ± 0.3 in control, 4.5 ± 0.5 in DSS-Control, 4.8 ± 0.2 in DSS-Lard, and 5.3 ± 0.5 in DSS-Fish; global P = 0.12). Mice were given regular tap water for the following 2 d before they were killed. Body weight and a symptom score (0 = no symptoms, 1 = blood at rectal area, 2 = bloody excrement, 3 = massive bloody diarrhea, 4 = animal deceased) were recorded daily for these last 4 d.

**Macroscopic scoring of colonic inflammation.** Mice were killed with a mixture of 95%/5% CO2/O2 (AGA) and by decapitation. The entire colon was dissected, opened, and flushed free of intestinal contents. The preparation was pinned onto a silicon dish and photographed. Three experienced evaluators independently scored the samples, without knowledge of the treatment groups, for a general inflamed appearance on a visual analogue scale. Before calculating the mean from the scores given by the 3 evaluators, scores were scaled so that the highest score for a single evaluator received the value 100 and others a value proportional to the highest one.

**Measurement of intestinal permeability.** All mice were killed between 1100 and 1400 h. Mice were fed-deprived overnight and gavaged in the morning with 500 mg/kg 4 kDa fluorescein isothiocyanate dextran at a 125-g/L water solution. After 4 h, blood was collected by decapitation. Serum was diluted 1:5 and fluorescence was measured with a Wallac Victor1420 Multilabel counter (Perkin Elmer). Diluted serum from a mouse gavaged with water only was used as blank and dextran concentrations were calculated from a standard curve.

Proximal colon was dissected and mounted into a Ussing chamber system (EasyMount, Physiologic Instruments). Chamber-halves were filled with 5 mL Ringer solution on each side (120 mmol/L NaCl, 5 mmol/L KCl, 25 mmol/L NaHCO3, 1.8 mmol/L Na2HPO4, 0.2 mmol/L NaH2PO4, 1.25 mmol/L CaCl2, 1 mmol/L MgSO4, and 10 mmol/L glucose). The system was water-jacketed to 37°C and carbonated with a carbogen (95% O2, 5% CO2, AGA) gas flow. After equilibration for 20 min, solutions were replaced with fresh Ringer, and sodium fluorescein (Sigma-Alrdich) was added to the luminal side to a final concentration of 2 µg/mL. Serosal fluorescence was detected at 45 and 60 min with a Wallac Victor1420 Multilabel counter and compared with luminal fluorescence. Data are expressed as percent translocated fluorescein.

**Biochemical analysis.** Jejunum and proximal colon were snap-frozen in liquid nitrogen and stored at −80°C. Serum was stored at −20°C. Tissue proteins were extracted as previously described (28). In short, samples were homogenized with Precellys24 (Bertin Technologies) at 77°C and briefly sonicated to ensure cell lysis in PBS with protease inhibitor (Complete mini, Roche). Homogenates were centrifuged for 5 min at 5000 × g (4°C) and the supernatant was again centrifuged 10 min at 10,000 × g (4°C). The final supernatant was stored at −80°C for analysis. The protein concentration was analyzed with a Pierce BCA Protein Assay kit (Thermo Scientific) and tissue TNFα was analyzed with a commercial kit (RBMS607Z/2R, Biovendor) in a single run with a CV of 4.3%. Data for tissue samples are expressed as pg/mg protein.

**Statistical analysis.** The primary variables were the fecal bile acids and hydrophobicity index measured during dietary intervention and the variables describing the effects of DSS (symptom score and intestinal permeability). The secondary variables were the variables measured during caging (food and water intake, and urine and fecal excretion) and the body weight before and after DSS. The choice between parametric and nonparametric statistical methods was based on the normality of variable distributions, which was checked using tests for normality (Kolmogorov-Smirnov test) and graphical plots. Also, the homogeneity of variances (Levene test) was assessed first. If the deviation from a normal distribution was only moderate, data were parametrically analyzed. The need to include baseline as a covariate also justified the parametric approach.

Fecal bile acid variables and hydrophobicity index were analyzed using ANOVA. Control and DSS-Control groups were combined (“Controls”), because the treatments of these groups did not differ at the time of bile acid analyses. DSS-Lard and DSS-Fish oil were called “High-fat (HF) Lard” and “HF Fish oil” before DSS induction.

The Kruskal-Wallis test was used to compare the study groups with respect to metabolic caging data. Body weight development during the dietary intervention at weeks 1–3 and days 1–2 after DSS was analyzed using repeated-measures ANCOVA. In both repeated-measures analyses, the baseline weights, week 0 before dietary intervention, and d 0 before DSS, respectively, were included as covariates.

The Kruskal-Wallis test was also used for the mean of d 4 symptom scores after DSS. The mean of symptom scores was calculated to summarize the well-being after the DSS intervention. In all analyses, the
Bonferroni-adjusted multiple comparisons were conducted only if the global $P$ value was significant. Independent samples $t$ tests and Mann-Whitney $U$ tests were used after significant ANOVA and Kruskal-Wallis test, respectively. The results are given as means ± SEs or medians (IQRs) for variables with normal and skewed distributions, respectively. Baseline-adjusted marginal means for weight development are given as means (95% CIs). Pearson or Spearman correlation coefficients were calculated when appropriate. $P$ values <0.05 were considered significant. IBM SPSS version 21.0 was used for statistical analyses.

**Results**

**Food consumption and body weight development.** During the first 3 wk of dietary intervention, the body differed ($P < 0.001$) between groups, and all comparisons between groups were significant ($P < 0.05$) (Fig. 1A). During metabolic caging, there were no significant differences in water intake, urinary excretion, or fecal excretion between the groups (Table 1). Due to the higher energy density of the HF diets, feed intake in the DSS-Lard and DSS-Fish oil groups was less than in the control and DSS-Control groups ($P < 0.05$), except the DSS-Fish oil group did not differ from the control group ($P = 0.07$). Protein intake was slightly lower ($P < 0.05$) in HF-lard–fed mice (median: 0.54 g/d; IQR: 0.51, 0.59) compared with controls (median: 0.69 g/d; IQR: 0.66, 0.79) or HF-fish oil-fed mice (median: 0.61 g/d; IQR: 0.59, 0.64).

**Total fecal bile acids.** The total fecal bile acid concentration was 71% greater in the HF Fish oil mice (4780 ± 340 μg/g) than in controls (2790 ± 280 μg/g; $P < 0.001$). The concentration in the HF Lard group (3600 ± 290 μg/g) did not differ from the controls but was 33% less than in the HF-Fish oil mice ($P = 0.04$). This was not explained by higher food intake, because fecal bile acid concentration was not correlated with fat intake in the mice fed the HF diets (Pearson’s $r = 0.03$; $P = 0.99$).

**Fecal bile acid concentrations.** The fecal concentration of cholic acid was 108% higher in HF Lard mice compared with controls ($P = 0.016$), but no other differences were significant (Table 2). There was a tendency toward higher concentrations of DCA and lithocholic acid in the HF Lard group compared with controls (+110%, $P = 0.09$ and +58%, $P = 0.052$, respectively). HF Fish oil mice had higher concentrations of these bile acids compared with controls ($P = 0.001$ for DCA, cholic acid, and lithocholic acid) as well as those of UDCA ($P = 0.028$) and $β$-muricholic acid ($P = 0.005$). Interestingly, the fecal DCA concentration was higher in HF Fish oil mice compared with HF Lard mice (+81%; $P = 0.006$). HF Fish oil mice also had a higher

### Table 1: Metabolic caging (48 h) data from mice at week 3

<table>
<thead>
<tr>
<th></th>
<th>Low-fat diets</th>
<th>HF diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DSS-Control</td>
</tr>
<tr>
<td>Food intake</td>
<td>2.9$^a$ (2.8, 3.6)</td>
<td>2.9$^b$ (2.6, 3.3)</td>
</tr>
<tr>
<td>Water intake</td>
<td>3.2 (3.0, 4.2)</td>
<td>3.1 (2.8, 3.3)</td>
</tr>
<tr>
<td>Urine excretion</td>
<td>0.61 (0.54, 1.19)</td>
<td>0.75 (0.61, 0.84)</td>
</tr>
<tr>
<td>Fecal excretion</td>
<td>0.26 (0.21, 0.33)</td>
<td>0.25 (0.23, 0.29)</td>
</tr>
</tbody>
</table>

$^a$ Data are expressed as medians (IQRs). Values without a common letter differ, $P < 0.05$; $n = 10$ in each group. DSS-Control, group fed control diet and colitis induced with dextran sodium sulfate; DSS-Fish oil, group fed a high-fat diet with lard and fish oil and colitis induced with dextran sodium sulfate; DSS-Lard, group fed a high-fat diet and colitis induced with dextran sodium sulfate; HF, high-fat.

$^b$ Kruskal-Wallis test.
concentration of UDCA compared with that of HF Lard mice (+50%; P = 0.029).

Fecal bile acid profile. The relative proportion of DCA was elevated in HF Fish oil mice (P = 0.001) but not in HF Lard mice (P = 0.11) (Table 2). However, dietary lard decreased the fecal UDCA concentration (P = 0.04). This led to a 76% higher fecal hydrophobicity index in the HF Lard group compared with controls (P = 0.051) and a 122% higher hydrophobicity index in the HF Fish oil group compared with controls (P = 0.001) (Fig. 2). The proportion of fecal DCA and the fecal hydrophobicity index did not differ between the HF Fish oil and HF Lard groups.

Development of experimental colitis. On day 2 of DSS administration, baseline-adjusted body weight was greater in controls (mean: 27.0 g; 95% CI: 26.4, 27.6 g) than in DSS-Control (mean: 25.7 g; 95% CI: 25.2, 26.3 g; P < 0.05) or DSS-Fish oil (mean: 25.3 g; 95% CI: 24.8, 25.9 g; P < 0.05) mice. The DSS-Lard group (mean: 26.0 g; 95% CI: 25.4, 26.5 g) did not differ from any other group (Fig. 1B). The general well-being deteriorated during DSS treatment and 60% of the mice died or had to be killed by d 4 after starting DSS, whereas all control mice were alive. The number of mice alive after d 2 is shown in Figure 1B. The prevalence of colitis symptoms (symptom score >0) was as follows: 0% control, 90% DSS-Control, 60% DSS-Lard, and 90% DSS-Fish oil. There were significant differences in mean symptom scores (Fig. 3): the DSS-Control and DSS-Fish oil groups had a higher mean symptom score compared with the healthy control (P < 0.001 for both comparisons), but the DSS-Lard group did not differ from control (P = 0.32) or any DSS groups. Similar results were obtained with the colonic inflammation score [median: 4; IQR: 0.6, 5.1 (for control, n = 8); median: 58; IQR: 56, 79 (for DSS-Control, n = 5, P = 0.005 vs. control); median: 27; IQR: 9.7, 63 (for DSS-Lard, n = 9; P = 0.021 vs. control, P = 1.0 vs. DSS-Control); 19 and 77 (for DSS-Fish oil, n = 2, no statistical testing performed)]. Despite macroscopically visible inflammation, TNFα concentrations in colon were lower in DSS groups [median: 51 pg/mg protein; IQR: 48, 51 (for DSS-Control); median: 36 pg/mg protein; IQR: 33, 40 (for DSS-Lard); n = 5–6 per group] compared with healthy controls (median: 78 pg/mg protein; IQR: 72, 85) (P < 0.01 for both comparisons).

Association between bile acids and colitis. The mean symptom score was correlated with fecal bile acid hydrophobicity in DSS mice (Spearman’s ρ = 0.43; P = 0.028; n = 26) (Fig. 4). DCA was the only bile acid that correlated with the symptom score for its concentration (Spearman’s ρ = 0.3; P = 0.048).

Intestinal permeability. Intestinal permeability could not be measured from all mice, because several mice died from the DSS treatment. Intestinal permeability in vivo and fluorescein permeability in the Ussing chamber did not differ between any of the groups (data not shown).

Discussion

The purpose of this experiment was to determine whether a diet with 60% E% lard or lard with fish oil affects susceptibility to DSS colitis via a potential mechanism related to luminal bile acids in a 4-wk feeding trial in mice. An index of fecal bile acid hydrophobicity was calculated, because this variable is closely linked with cytotoxicity.

In the present study, we demonstrate that fecal bile acid hydrophobicity is positively correlated with the severity of DSS colitis symptoms. There are several possible mechanisms by which bile acids may alter the pathogenesis of DSS colitis. One of these is related to the higher proportion of hydrophobic bile acids after HF compared with low-fat feeding, as also shown in this study. Hydrophobic bile acids impair gut barrier function, in DSS mice (Spearman’s ρ = 0.43; P = 0.028; n = 26) (Fig. 4).

**TABLE 2** Fecal bile acid concentrations and profile from controls and HF-fed mice after 3 wk of feeding before induction of colitis

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Controls</th>
<th>HF Lard</th>
<th>HF Fish oil</th>
<th>Global P</th>
<th>% of all fecal bile acids</th>
<th>% of all fecal bile acids</th>
<th>% of all fecal bile acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/dry feces</td>
<td>g/dry feces</td>
<td>g/dry feces</td>
<td>Global P</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCA</td>
<td>10.0 ± 1.0</td>
<td>7.4 ± 0.6</td>
<td>8.8 ± 0.8</td>
<td>0.10</td>
<td>0.38 ± 0.03a</td>
<td>0.23 ± 0.03b</td>
<td>0.19 ± 0.02b</td>
</tr>
<tr>
<td>ECA</td>
<td>58.8 ± 7.1b</td>
<td>88.0 ± 8.3b</td>
<td>111 ± 11.9a</td>
<td>0.001</td>
<td>2.1 ± 0.2</td>
<td>2.5 ± 0.3</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>HCA</td>
<td>90.4 ± 17.1</td>
<td>134 ± 16.4</td>
<td>144 ± 20.2</td>
<td>0.09</td>
<td>3.0 ± 0.5</td>
<td>3.8 ± 0.4</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>DCA</td>
<td>254 ± 34b</td>
<td>533 ± 64b</td>
<td>985 ± 144a</td>
<td>&lt;0.001</td>
<td>9.3 ± 1.2b</td>
<td>15.2 ± 2.1b</td>
<td>20.3 ± 2.5a</td>
</tr>
<tr>
<td>CDCA</td>
<td>21.4 ± 2.1</td>
<td>26.0 ± 2.1</td>
<td>29.4 ± 2.9</td>
<td>0.07</td>
<td>0.85 ± 0.11</td>
<td>0.74 ± 0.08</td>
<td>0.63 ± 0.06</td>
</tr>
<tr>
<td>CA</td>
<td>242 ± 39b</td>
<td>503 ± 69b</td>
<td>596 ± 80a</td>
<td>0.001</td>
<td>8.1 ± 0.7b</td>
<td>13.9 ± 1.9a</td>
<td>12.3 ± 1.3b</td>
</tr>
<tr>
<td>UDCA</td>
<td>10.9 ± 1.4b</td>
<td>10.7 ± 1.1b</td>
<td>16.0 ± 1.3b</td>
<td>0.01</td>
<td>0.39 ± 0.03b</td>
<td>0.29 ± 0.02b</td>
<td>0.34 ± 0.02a</td>
</tr>
<tr>
<td>β-MCA</td>
<td>130 ± 16b</td>
<td>184 ± 25b</td>
<td>227 ± 19a</td>
<td>0.006</td>
<td>5.0 ± 0.6</td>
<td>5.0 ± 0.5</td>
<td>4.8 ± 0.2</td>
</tr>
</tbody>
</table>

1 Data are expressed as means ± SEMs, n = 12 (controls) or 10 (HF groups). Groups in a row without a common letter differ, P < 0.05. CA, cholic acid; CDCA, 254 deoxycholic acid; ECA, 254 epideoxycholic acid; HCA, 254 hydroxycholic acid; DCA, 254 deoxycholic acid; UDCA, 254ursodeoxycholic acid; β-MCA, β-muricholic acid. ANOVA with Bonferroni-corrected multiple comparisons was conducted.

**FIGURE 2** Fecal bile acid hydrophobicity index before DSS administration of mice fed a low-fat, control diet or HF diets containing lard or fish oil for 3 wk. Feces were analyzed with GC. Hydrophobicity index was calculated as a percentage-weighted mean of individual bile acid hydrophobicities. Bars show means ± SEMs, n = 12 for controls and 10 for HF groups. Labeled means without a common letter differ, P < 0.05 (ANOVA with Bonferroni-corrected multiple comparisons). *P = 0.051 compared with controls. DSS, dextran sodium sulfate; HF, high-fat.
In contrast to hydrophilic bile acids, which may protect the gut from barrier dysfunction (3), the translocation of these microorganisms across the tight-junctional barrier seems important in the onset of disease. Increased fecal bile acid hydrophobicity may prime the gut to experimental colitis by facilitating the translocation of luminal microbes, or their structures.

Araki et al. (30) reported that a lower concentration of fecal cholic acid was related to higher macroscopic damage, although DSS administration increased the proportion of cholic acid in feces. We noted no such correlation. As recently reported (31), selected types of dietary fat may promote colitis also via alterations in gallbladder bile and gut microbiota. Milk fat increased the incidence of colitis, which was suggested to result from the higher proportion of taurocholic acid in gallbladder bile and facilitated the growth of the inflammatory Bilophila wadsworthia (31). It is likely that there are also numerous other mechanisms, only some of them related to bile acids, by which dietary fat affects susceptibility to colitis.

To our knowledge, this is the first study to investigate the effect of dietary fat on fecal bile acid hydrophobicity index instead of only comparing concentrations of individual bile acids. These data demonstrate that in a short-term intervention, only a HF diet with fish oil results in higher fecal bile acid hydrophobicity compared with a low-fat diet, whereas there is only a tendency for higher fecal hydrophobicity after lard feeding. The higher hydrophobicity index in the fish oil group seemed to be mostly mediated through a high proportion of deoxycholic acid. A study by Reddy et al. (23) compared fish oil with corn oil in a 12-wk study. In contrast to our results, they reported a lower concentration of secondary bile acids by fish oil compared with corn oil, which was reflected as a lower 7α-dehydroxylase activity in fish oil-fed mice. Fish oil was the primary source of fat in their study diet, comprising 20.5% of total weight of menhaden oil, whereas our diet contained 15.8% of total weight menhaden oil with an equal amount of lard. It may be suspected that differences in study diets could affect outcome. Moreover, because 7α-dehydroxylase is an enzyme formed by the gut microbiota to convert primary bile acids into their secondary forms, results may be affected by the initial microbiome of the study animals.

After 3 wk of dietary intervention, the fecal bile acid concentration was significantly elevated by fish oil, but not by dietary lard. Our previous study demonstrated an elevated bile acid concentration in mice fed a HF lard diet after 13 wk of feeding (11). Because bile acid excretion was slightly but not significantly, higher in lard-fed mice compared with controls, it may be postulated that increased bile acid excretion by lard requires a longer duration of intervention.

In the present study, the fecal bile acid concentration was higher after fish oil feeding than lard feeding. Earlier studies have not reached a conclusion on whether fat quality affects total bile acid excretion. Studies by Reddy et al. (12,23) found no difference when comparing corn oil with lard and corn oil with fish oil after 11–12 wk of feeding in rats. Sato et al. (21) compared different fat qualities in rats and demonstrated a substantially higher excretion of bile acids by the saturated fats lard and tallow, whereas the opposite was noted for sesame oil. Fish oil was not included in the study. In humans, Connor et al. (32) showed that bile acid excretion was higher after feeding corn oil compared with cocoa butter, but the opposite was reported in mice (22). There seems to be no consensus on how dietary fat quality affects fecal bile acid excretion. The higher fecal bile acid concentration in mice consuming fish oil compared with those consuming lard, as reported here, cannot be affirmatively explained by a higher concentration of luminal lipids despite higher food intake, because bile acid concentration was not correlated with fat intake. As Connor et al. (32) have suggested, increased excretion of bile acids may be related to decreased plasma cholesterol concentrations. This may explain the difference in bile acid excretion in the present study, although we did not measure serum cholesterol to verify this.
To our surprise, the mice in this study developed very severe colitis after only 2 d of DSS in drinking water. We previously used the 5% dose of DSS in our laboratory successfully as a 5-d protocol, combined with a regular chow diet. The diets in the present study contained much less fiber, which may have an effect on the efficacy of DSS on the intestinal epithelium. It is also possible that dietary fish oil, which naturally decreases blood clotting, increased gut epithelial bleeding in the DSS-Fish oil group and may have confounded data for colitis symptoms.

Previous reports have demonstrated varying effects of fish oil on susceptibility to experimental colitis. Whereas some studies have shown that fish oil supplementation (5% wt:wt) reduces DSS-induced weight loss and DNA damage (33) or that other n–3 (ω3) oils ameliorate inflammation in the same model (34,35), several studies report the opposite. Fish oil, varying between 0.75 and 8% wt:wt, has severely exacerbatied colitis scores in several colitis models: DSS colitis (36), the IL-10 knockout mouse (37), and the H. hepaticus-induced colitis model (38), in which fish oil resulted in a dose-dependently higher intestinal histopathological score. It is unclear whether this effect is inflammation mediated. It seems, however, that high doses of fish oil may adversely affect experimental colitis. Fiber sources have been reported to bind bile acids differently (39), which may affect development of colitis during a fish oil diet. The only study reporting a preventive effect for fish oil (33) used a purified diet containing cellulose, similarly to the present study. Of the other 3 studies reporting exacerbation by fish oil (36–38), 2 used a purified diet and 1 used regular chow, which contains plant fiber. Thus, the fibers used in the studies on fish oil in DSS colitis do not seem to explain differences in results.

It is notable that a transgenic mouse model capable of producing n–3 fatty acids from n–6 fatty acids has a reduced severity of DSS colitis (40). These mice have a tissue n–6:n–3 ratio of 1.66 compared with 30.13 in wild-type mice. A diet containing 6% fish oil and 1% corn oil has an n–6:n–3 ratio of 0.17 and has been reported to exacerbate colitis (38). Although tissue and dietary ratios are difficult to compare, it is possible that a very high dose of fish oil is detrimental in colitis, whereas a moderate dose is protective. In the present study, we used a much higher dose of fish oil (27.2% kcal) compared with all previous studies on fish oil and colitis (17.2% kcal) (33,36–38). However, because we did not see any significant effect from fish oil, this difference does not explain discrepancies among previous studies. In contrast, the lack of effect, positive or negative, in the present study could be explained by the short dietary intervention (3 wk compared with 5–8 wk in previous studies) or high severity of colitis.

Several mice in all DSS groups died before the study endpoint and many were dehydrated at the time of killing. Thus, intestinal permeability could be measured in only a few mice. These data may be biased by the lack of those mice that prematurely died. Perhaps due to this reason there was no difference in gut permeability between healthy controls and mice with DSS colitis. Moreover, in the in vivo method of measuring permeability, probes are measured from the circulation. When mice are dehydrated and blood volume decreases, there may be bias in these data. Therefore, data on the effect of dietary fat on intestinal permeability cannot be reliably interpreted in this study.

In summary, we demonstrate here that fecal bile acid hydrophobicity is correlated with symptom severity in DSS mice. These data also suggest that dietary fat does not alter susceptibility to DSS colitis but causes unbeneficial modifications in the fecal bile acid profile.

Acknowledgments

The authors are most grateful to Leena Kaipainen for performing all bile acid analyses, statistician Tuuja Poussa for help with statistical analyses, and Professor (emeritus) Heikki Vapaatalo for insightful comments on the study design. They also thank Hanna Keränen for help with mouse work. L.K.S., R.H., H.G., and R.K. designed research; L.K.S. and R.F conducted research; H.G. provided bile acid analyses; L.K.S. and R.F. analyzed data; L.K.S. wrote the paper; and L.K.S. had primary responsibility of the final content. All authors read and approved the final manuscript.

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Dietary fat, bile acids, and experimental colitis


