Laxative treatment with polyethylene glycol decreases microbial primary bile salt dehydroxylation and lipid metabolism in the intestine of rats

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1Top Institute Food and Nutrition, Wageningen, The Netherlands; 2Pediatric Gastroenterology and Hepatology, Department of Pediatrics, Beatrix Children’s Hospital, Groningen University Institute for Drug Exploration, Center for Liver, Digestive and Metabolic Diseases, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands; 3Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands

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van der Wulp MY, Derrien M, Stellaard F, Wolters H, Kleerebezem M, Dekker J, Rings EH, Groen AK, Verkade HJ. Laxative treatment with polyethylene glycol decreases microbial primary bile salt dehydroxylation and lipid metabolism in the intestine of rats. Am J Physiol Gastrointest Liver Physiol 305: G474–G482, 2013. First published July 18, 2013; doi:10.1152/ajpgi.00375.2012.—Polyethylene glycol (PEG) is a frequently used osmotic laxative that accelerates gastrointestinal transit. It has remained unclear, however, whether PEG affects intestinal functions. We aimed to determine the effect of PEG treatment on intestinal sterol metabolism. Rats were treated with PEG in drinking water (7%) for 2 wk or left untreated (controls). We studied the enterohepatic circulation of the major bile salt (BS) cholate with a plasma stable isotope dilution technique and determined BS profiles and concentrations in bile, intestinal lumen contents, and feces. We determined the fecal excretion of cholesterol plus its intestinally formed metabolites. Finally, we determined the cytolytic activity of fecal water (a surrogate marker of colorectal cancer risk) and the amount and composition of fecal microbiota. Compared with control rats, PEG treatment increased the pool size (+51%; P < 0.01) and decreased the fractional turnover of cholate (−32%; P < 0.01). PEG did not affect the cholate synthesis rate, corresponding with an unaffected fecal primary BS excretion. PEG reduced fecal excretion of secondary BS and of cholesterol metabolites (each P < 0.01). PEG decreased the cytolytic activity of fecal water (54 [46–62] vs. 87 [85–92]% erythrocyte potassium release in PEG-treated and control rats, respectively; P < 0.01). PEG treatment increased the contribution of Verrucomicrobia (P < 0.01) and decreased that of Firmicutes (P < 0.01) in fecal flora. We concluded that PEG treatment changes the intestinal bacterial composition, decreases the bacterial dehydroxylation of primary BS and the metabolism of cholesterol, and increases the pool size of the primary BS cholate in rats.

cholate; enterohepatic circulation; bile salt; microbiota

POLYETHYLENE GLYCOL (PEG) presently is one of the most widely prescribed laxatives. Long-term treatment with PEG is believed to be safe and highly effective (1, 27). However, it is not known whether or not long-term PEG treatment affects specific intestinal metabolic functions. PEG may change the intestinal milieu by accelerating the passage of luminal contents and/or by increasing the luminal water content, possibly leading to a change in the intestinal microflora (10).

We have previously shown that PEG treatment accelerates the transit through the small intestine as well as the whole gut in rats (10). PEG decreases fecal excretion of bile salts (BS) in rats (10). BS facilitate the solubilization of lipids (dietary fats, cholesterol, and fat-soluble vitamins) in the small intestine, which is required for efficient lipid absorption (41). It has recently become clear that BS not only are detergents necessary for lipid absorption but also are involved in the regulation of glucose and lipid homeostasis and energy expenditure (22, 26). This is illustrated, for example, by the fact that removal of BS from the intestine with sequestrants improves plasma low-density lipoprotein levels and hyperglycemia in patients with type II diabetes (5, 6). The mechanisms by which the total pool size and/or profile of BS influence different physiological processes are just beginning to be understood (22, 26).

BS are amphiphatic molecules, produced in the liver (primary BS) by catabolism of cholesterol (16). The liver conjugates BS with taurine or glycine and secretes BS into bile, which is transported to the small intestine (31). Lipid absorption mainly takes place in the proximal small intestine, whereas BS are actively reabsorbed with high efficiency (~95%) in the terminal ileum (31). From there, they are transported back to the liver through the portal system, i.e., completing their enterohepatic cycle (EHC). Under steady-state conditions, the liver compensates for fecal BS loss by synthesis of new BS, which then are secreted into bile.

The small percentage of BS that escapes absorption in the terminal ileum enters the colon. Intestinal microbiota deconjugate (through BS hydrolases) and dehydroxylate (7α-dehydroxylase) primary BS to so-called secondary BS species (31). A part of colonic BS is passively absorbed, whereas the remaining part is lost with feces. Secondary BS such as deoxycholate (DC) and lithocholate (LC) are highly hydrophobic. It is generally thought that these hydrophobic BS can damage colonic mucosal cells and play a role in initiating or propagating the formation of gastrointestinal malignancies (3, 31). In addition, an increased hydrophobic BS pool size is associated with the formation of cholesterol gallstones (4, 24, 35).
Based on our previous studies (10), we hypothesized that PEG disrupts the EHC of BS by changing the intestinal milieu. We determined relevant parameters of the EHC of cholate (quantitatively the major BS in humans and rodents) during PEG treatment by an isotope dilution technique (17, 18). We determined the effects of PEG on BS profiles and concentrations in different compartments of the EHC, on the cytotoxic activity of fecal water, and on fecal microbiota. This study shows that PEG changes the microbial composition, decreases secondary BS formation, and increases the cholate pool size. Furthermore, PEG treatment leads to decreased fecal water cytotoxicity, which could possibly indicate increased health of colonic cells.

MATERIALS AND METHODS

Materials

Colofort (polyethylene glycol + electrolytes) was obtained from Ipsen Farmaceutica (Hoofddorp, The Netherlands). Colofort contained per sachet (74 g): 64 g PEG, molecular weight 4 kDa, 5.7 g sodium sulfate (anhydric), 1.68 g sodium bicarbonate, 1.46 g sodium chloride, and 0.75 g potassium chloride. 24-13C-cholate was obtained from Cambridge Isotope Laboratories (Andover, MA) and was of 98–99% isotopic purity. Complete protease inhibitor was obtained from Roche (Indianapolis, IN). Deuterium labeled D7-7α-hydroxycholesterol (7α-hydroxycholesterol-25,26,26,27,27,27-D7) was obtained from CDN Isotopes (cat. no. D-4064; Essex, UK).

Animals

Outbred male Wistar Unilever rats (150–174 g) were obtained from Harlan (Horst, the Netherlands). Rats were housed individually in an environmentally controlled facility with diurnal (12-h/12-h) light/dark cycle. They were maintained on semisynthetic purified diet (low-fat normal calcium food; cat. no. 4063.02; Arie Blok, Woerden, the Netherlands). Before any intervention, rats were accustomed to the diet during a 3-wk run-in period. Food and water were available ad libitum during the entire study period. The experiments were performed in conformity with public Health Service policy and with national laws. The ethics Committee for Animal Experiments of the University Medical Center of Groningen approved the experimental protocols.

Cholate Kinetic Study

After the run-in period on semisynthetic diet, rats were randomly assigned to either control group (no treatment; n = 7) or intervention group (71 g/1 PEG4000 by drinking water; n = 7) for a total period of 16 days. Feces were collected during a period of 48 h before the start of treatment and again after 1 wk of PEG treatment. Food and fluid intake and body weight were measured daily. Some of the rat characteristics have been reported in our previous paper, which reported on the aspects of PEG treatment on fat and cholester absorption (37).

Cholate kinetics. Intravenous 24-13C-cholate (3 mg per rat in a solution of 250 μl 0.5% NaHCO3 in PBS, pH 7.4) was administered at day 10 after the start of PEG treatment. Blood was drawn from the tail vein at 0, 12, 24, 36, 48, and 60 h after administration under isoflurane anesthesia. At day 17, rats were anesthetized by intraperitoneal injection of a mixture of Hypnorm (fentanyl/fluanisone, 1 ml/kg) and diazepam (10 mg/kg). The common bile duct was cannulated for bile collection. To ensure that hepatic production was accurately measured, bile produced during the initial 5 min after cannulation was discarded, and bile was sampled for 30 min thereafter. During the bile-collection period, body temperature was maintained by keeping animals in a humidified incubator. Blood was obtained by cardiac puncture and stored (−20°C). Rats were terminated by cervical dislocation.

Analytical procedures and calculations. FECES. BS and neutral sterol composition and concentration in feces were determined by gas chromatography (GC) to calculate daily excretion (38).

CHOLATE KINETICS. Plasma and bile samples were prepared for isotopic analysis of cholate α-bromopentafluorotoluene (PFB)-trimethylsilyl (TMS) derivatives by GC-mass spectrometry (MS) (18). The ions monitored were 623 and 624 corresponding to cholate (M0) by 10.2 ± 0.3 nmol/100 g body wt per h by the biliary cholate concentration (mM) as determined by GC analysis (38). Bile flow was determined gravimetrically, assuming a density of 1 g/ml for bile. The percentage of cholate reabsorbed per day was calculated as follows: 100% × (biliary secretion − synthesis)/biliary secretion. The percentage of cholate lost in feces per enterohepatic cycle was calculated by 100% minus the calculated percentage of reabsorption (17).

Hepatic Cholesterol 7 α-Hydroxylase (Cytochrome P-450 7a1 or cyp7a1) Activity

The activity of microsomal cyp7a1 was assayed essentially as described (14) with some modifications. In short, microsomes (200 μg protein) in 100 mM K-phosphate buffer, pH 7.4 containing 1 mM EDTA, 1 mM NADPH, and complete protease inhibitor were incubated at 37°C (total volume 1 ml). After 4 min, the enzymatic reaction was stopped by addition of 8 ml of chloroform/methanol (2:1; vol/vol). D7-7α-hydroxycholesterol was added as internal standard (40 ng). After adding 1 ml 0.9% NaCl and vigorous shaking, we centrifuged the samples. The chloroform phase was removed and evaporated under a stream of nitrogen. Calibration standards with unlabeled D7-7α-hydroxycholesterol were treated in a similar way. Samples were injected to TMS ether by overnight treatment with a mixture of N,O-bis (TMS) trifluoroacetamide, pyridine and trimethylchlorosilane (50:50:1, vol/vol). Samples were dried under a stream of nitrogen, resuspended in hexane, and analyzed on GC-MS (Agilent 9575C inert MSD; Agilent Technologies, Amstelveen, the Netherlands). The column used was J+W Scientific DB-17MS, 20 m, 0.18 mm, film 0.18 μm. Operating parameters were as follows: helium flow 0.7 ml/min, temperature source 220°C, column 150°C for 0.5 min-60°C/min up to 300°C for 5 min. The amount of 7α-hydroxycholesterol produced from the endogenous microsomal cholesterol was calculated from the ratio labeled/unlabeled 7α-hydroxycholesterol using the calibration standards.

Cytotoxic Activity of Fecal Water

Fecal water was prepared by reconstitution of lyophilized feces, which also allowed for estimation of fecal dry and wet weight percentage of the original feces (33). Potassium release resulting from lysis of human erythrocytes incubated in 154 mM NaCl was set as 0%, and distilled water represented 100% (maximal) potassium release. Subsequently, fecal water was incubated with erythrocytes, and potassium release as a measure of cell lysis was expressed as percentage of maximal potassium release (15).

Intestinal BS Contents

BS in intestinal contents were measured in a separate group of rats, treated and handled identical to described above; rats were randomly
assigned to control group or PEG-treated group (n = 7 per group). At day 16 after the start of PEG treatment, rats were terminated under anesthesia, and the entire intestinal tract from duodenum until anus was removed. Small intestine was separated from colon. Cecum was separated from remaining colon, and its contents were collected directly in preweighed cups. Small intestine and remaining colon were flushed with 5–15 mL PBS to collect contents. Samples were lyophilized overnight and weighed again to calculate total dry content weight. Aliquots of 50 mg were used to determine BS composition by GC. BS were first isolated by reversed-phase solid-phase extraction (38). This way, we obtained the total fractions of all BS in the different compartments.

**Bacterial DNA Extraction**

Fecal bacterial genomic DNA was isolated using the Fast DNA Spin kit (Qiobioengineering, Carlsbad, CA) using 0.1 g of fecal sample and eluted in 100 μL DES. Purity and amount of DNA was measured using Nanodrop-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

**Quantitative PCR**

Quantitative PCR (qPCR) was performed with an IQ5 Cycler apparatus (Bio-Rad, Veenendaal, The Netherlands). All reactions were performed in triplicate in one run. Samples were analyzed in a 25-μL reaction mix consisting of 12.5 μL Bio-Rad master mix SYBR Green (50 mM KCl, 20 mM Tris-HCl, pH 8.4, 0.2 mM of each dNTP, 250 μL of template DNA). The following conditions of qPCR used were 95°C for 15 s, annealing temperature of 60°C for 20 s, extension at 72°C for 70 s, and a final extension step at 72°C for 5 min. The amplification program consisted of an initial denaturation step at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 40 s, elongation at 72°C for 70 s, and a final extension step at 72°C for 10 min. The size of the PCR products was confirmed by gel electrophoresis using 1 μL of the reaction mixture on a 1% (wt/vol) agarose gel containing ethidium bromide. DNA yield from purified PCR products was measured by Nanodrop ND-1000 spectrophotometer. Pooled purified PCR (final DNA concentration of 100 ng/μL) was subsequently sent to GATC-Biotech for pyrosequencing using a Genome Sequencer FLX in combination with titanium chemistry.

**16S rRNA gene sequence analysis.** 16S rRNA gene sequences generated from pyrosequencing were quality filtered. Sequences were removed if they were shorter than 200 nucleotides, longer than 1,000 nucleotides, or contained primer mismatches or ambiguous bases. The remaining sequences were analyzed using the open-source software package Quantitative Insights Into Microbial Ecol-ogy (QIIME) (7). 16S rRNA gene sequences were assigned to operational taxonomic units (OTUs) using UCLUST with a threshold of 97% pairwise identity, then classified taxonomically using the Ribosomal Database Project classifier 2.0.1. Results were displayed as relative abundance of bacterial taxa and richness.

**Statistical Analyses**

Normal distribution was examined by Kolmogorov-Smirnov and Shapiro-Wilk tests and normal probability plots. Nonparametrically distributed data were tested for significant differences by Mann Whitney U-test (values represent median and interquartile range) and parametrically distributed data by Student’s t-tests.

Table 1. Animal characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PEG</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>398 ± 32</td>
<td>391 ± 44</td>
</tr>
<tr>
<td>Food intake, g/kg body wt per day</td>
<td>59 ± 2</td>
<td>58 ± 4</td>
</tr>
<tr>
<td>Fluid intake, g/kg body wt per day</td>
<td>67 ± 13</td>
<td>79 ± 12</td>
</tr>
<tr>
<td>Dry feces output, g/kg body wt per day</td>
<td>8.0 ± 0.8</td>
<td>11.4 ± 1.1*</td>
</tr>
<tr>
<td>Fecal water output, g/kg body wt per day</td>
<td>9.3 ± 1.6</td>
<td>20.2 ± 2.5*</td>
</tr>
<tr>
<td>Total fecal output, g/kg body wt per day</td>
<td>17.3 ± 1.6</td>
<td>31.6 ± 3.2*</td>
</tr>
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</table>

Data are presented as means ± SD, n = 7 per group. Data of polyethylene glycol (PEG)-treated rats were compared with those of control rats by unpaired 2-sided Student’s t-tests. *P < 0.0001.

16S rRNA Gene Amplicon Pyrosequencing

Amplicons from the V1-V3 region of 16S rRNA genes were generated by PCR using 27F-Deg5′ (5′-GTGYGATMYTGTTGTTCAG-3′) in combination with 520R-Deg for 14 fecal samples. To facilitate pyrosequencing using titanium chemistry, each forward primer was appended with the titanium adaptor A (5′-CCTGTCCTACGACTCAG-3′) and a ‘NNNN’ barcode sequence on the 5′ end, where NNNN is a sequence of four nucleotides that was unique for each sample. The reverse primer carried the titanium adaptor B (5′-BioTEG/CCTATCCCTGTTGTGCTTGGCAGTCT-CAG-3′) on the 5′ end. Sequencing was performed from adaptor A. Adaptor and barcode sequences were provided by GATC Biotech (www.GATC-Biotech.com). PCRs were performed in a total volume of 50 μL containing 1× PCR buffer, 1 μL PCR Grade Nucleotide Mix, 0.4 μL of Faststart Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany), 200 nM of a forward and the reverse primer (Biologio, Nijmegen, Netherlands), and 20 of template DNA. The amplification program consisted of an initial denaturation step at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 40 s, elongation at 72°C for 70 s, and a final extension step at 72°C for 10 min. The size of the PCR products was confirmed by gel electrophoresis using 1 μL of the reaction mixture on a 1% (wt/vol) agarose gel containing ethidium bromide. DNA yield from purified PCR products was measured by Nanodrop ND-1000 spectrophotometer. Pooled purified PCR (final DNA concentration of 100 ng/μL) was subsequently sent to GATC-Biotech for pyrosequencing using a Genome Sequencer FLX in combination with titanium chemistry.

**Table 2. Bile flow and biliary output parameters**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PEG</th>
</tr>
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<tbody>
<tr>
<td>Bile flow, ml/100 g body wt per day</td>
<td>6.5 [5.7–6.9]</td>
<td>6.2 [5.5–6.5]</td>
</tr>
<tr>
<td>BS concentration, mM</td>
<td>22.7 [19.3–25.0]</td>
<td>19.8 [16.2–23.3]</td>
</tr>
<tr>
<td>Cholate concentration, mM</td>
<td>8.3 [6.3–12.7]</td>
<td>10.2 [6.9–13.0]</td>
</tr>
<tr>
<td>Biliary BS secretion rate, μmol/100 g body wt per day</td>
<td>133.0 [112.1–172.5]</td>
<td>117.5 [101.7–132.0]</td>
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</table>

Bile flow was measured during a 30-min collection period. Bile salt (BS) secretion rate was calculated from total biliary BS concentration as determined by gas chromatography. Data are presented as median and interquartile range, n = 6–7 per group. Data of PEG-treated rats were compared with those of control rats by Mann Whitney U-tests.
t-test (values represent means ± SD). Because some of the data regarding BS kinetics and sterols in intestinal lumen contents and feces were clearly nonparametrically distributed, we decided to test all these data nonparametrically to allow for comparison between graphs. Statistical analyses were performed using SPSS 18.0 for Windows (Chicago, IL) and GraphPad Prism (San Diego, CA). Differences between groups were considered statistically significant at \( P < 0.05 \).

**RESULTS**

**Animal Characteristics**

PEG-treated rats did not differ from control rats in terms of body weight, food intake, or fluid intake. Fecal dry weight as well as estimated fecal wet weight and total daily fecal (dry + wet) output were increased in PEG-treated compared with control rats (Table 1).

**PEG Treatment Results in Major Changes of the Enterohepatic Circulation of Cholate**

To determine the effects of PEG on the EHC of BS, we studied the kinetics of cholate, representing the major BS in human as well as rodent BS pools. PEG-treated rats had a similar bile flow and total biliary BS concentration and secretion rate compared with control rats (Table 2). Cholate kinetic parameters are schematically summarized in Fig. 1, A and B. PEG did not change biliary secretion rate or intestinal reabsorption (~96%) of cholate or the cholate synthesis rate. The latter finding corresponded with similar Cyp7a1 (rate-limiting

![Fig. 1](http://ajpgi.physiology.org/)

Fig. 1. Cholate kinetics (A and B). Biliary secretion, intestinal reabsorption, fecal excretion, and synthesis of cholate did not differ between control (A) and polyethylene glycol (PEG)-treated (B) rats. Cholate pool size, on the other hand, was increased during PEG treatment in the presence of reduced fractional turnover rate (FTR). Plasma cholate kinetics were studied during 60 h after intravenous administration of stable isotope-labeled \(^1\)C-cholate. If not indicated, values are expressed in \( \mu \text{mol}/100 \text{ g body wt per day} \). Data are presented as median and interquartile range, \( n = 6–7 \) per group. Data of PEG-treated rats were compared with those of control rats by Mann Whitney \( U \)-tests. **\( P < 0.01 \). As an indicator of total bile salt (BS) synthesis, Cyp7a1 activity was measured in liver microsomes. C: data are presented as means ± SD, \( n = 6–7 \) per group. Data of PEG-treated rats were compared with those of control rats by unpaired Student’s \( t \)-test.

AJP-Gastrointest Liver Physiol • doi:10.1152/ajpgi.00375.2012 • www.ajpgi.org
enzyme for BS synthesis) activity in livers of PEG-treated and control rats (Fig. 1C).

PEG treatment increased the cholate pool size (increase in median value +51%; \(P = 0.008\)) and decreased the FTR by 32% (\(P = 0.003\)). PEG did not change fecal primary BS excretion but decreased fecal secondary BS excretion by 46% [2.0 (1.6–2.6) vs. controls 3.6 (3.1–4.1) \(\mu\text{mol/100 g body wt per day}\), respectively; \(P = 0.001\); Fig. 2, A and B].

**PEG Treatment Alters Intestinal BS Composition**

Considering the kinetics of the enterohepatic circulation, we investigated whether the increased cholate pool size would be reflected by higher cholate amounts in the intestinal luminal contents. PEG treatment did not significantly affect the cholate content in the lumen of either the small intestine or the colon but increased it in the lumen of the cecum (Fig. 3).

**PEG Treatment Decreases Lipid Metabolites**

The unaffected primary BS and decreased secondary BS excretion via the feces could be due to altered bacterial deconjugation and conversion of primary BS. In agreement with this, PEG-treated rats had higher amounts of primary BS in cecum and total intestinal lumen contents (the latter not statistically significant; \(P > 0.07\); Fig. 4A). On the other hand, PEG-treated rats had lower amounts of secondary BS (Fig. 4B) in their intestinal lumen contents.

The data corresponded with those found in bile, in which we also found decreased secondary BS (Fig. 4, C and D). To determine whether the decreased microbial conversion upon PEG treatment was specific for BS metabolism, we analyzed fecal neutral sterols and SCFA. In accordance with the BS data, we found almost no cholesterol metabolites (Fig. 4E) or SCFA (Fig. 4F) in feces.

**PEG Treatment Decreases Fecal Water Cytotoxic Activity**

The aqueous, rather than the solid, phase of feces is considered to contain the compounds that interact with (large) intestinal mucosal cells (28). In our study, the cytotoxic activity induced by fecal water was significantly decreased in PEG-treated compared with control rats [54 (46–62) vs. 87 (85–91) % erythrocyte K release, respectively; Fig. 5; \(P = 0.001\)].

**PEG Treatment Changes Intestinal Microbiota Profile**

The PEG-induced differences in intestinal BS and sterol metabolite contents suggested alterations in the amount and/or activity of the intestinal microflora. Quantitative PCR of 16S amplicon showed that PEG-fed rats harbored a nonsignificantly lower bacterial load expressed as Log10 16SrRNA copies/g wet feces (\(P = 0.053\), Fig. 6A; 11.4 ± 0.1 vs. 11.0 ± 0.10 in control and PEG-fed rats, respectively), which is likely due to increased fecal water content. Microbiota composition of both groups was investigated by 454-pyrosequencing of the V1-V2 variable region. A total of 192,158 sequences were obtained.

After quality filtering, the average of sequence size was 314.6 (min: 200, max: 551.0). The number of reads per sample was on average 13,725. PEG treatment increased Verrucomicrobia (\(P = 0.006\)) but did not change other phyla significantly (Fig. 6B). With regard to lower taxonomical level, multivariate analysis on relative abundance of bacterial taxa (genus level) showed that microbiota from PEG-treated rats differ significantly from that of control rats (Monte Carlo permutation test, \(P = 0.004\), Fig. 6C).

Significant changes of genus level included mostly increased relative abundance of known mucus associated bacteria (Akermania, Bacteroides, Ruminococcus), members of Proteobacteria, and concomitant decreased relative abundance of Clostridia (Firmicutes) (Fig. 6, D and E). Moreover, the total

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**Fig. 2.** Excretion of individual BS (A) and primary vs. secondary BS (B) in feces. Primary BS include chenodeoxycholate (CDC), cholate (C), allocholate (alloC), \(\alpha\) and \(\beta\)-muricholate (MC). Secondary BS include ursodeoxycholate (UDC), deoxycholate (DC), hyodeoxycholate (HDC), and \(\alpha\)-MC. Fecal BS excretion was determined in 48-h-collected feces on days 8 and 9 after the start of PEG treatment. Data are presented as median and interquartile range, \(n = 6–7\) per group. Data of PEG-treated rats were compared with those of control rats by Mann Whitney U-tests. \(*P < 0.05, **P < 0.01\).

**Fig. 3.** Cholate amount in different intestinal compartments. SI, small intestine. Data are presented as median and interquartile range, \(n = 6–7\) per group. Data of PEG-treated rats were compared with those of control rats by Mann Whitney U-tests. \(*P < 0.05\).

**Fig. 4.** BS content in enteric compartments. SI, small intestine. Primary BS include CDC, C, alloC, \(\alpha\)-MC, \(\beta\)-MC, DC, HDC, and \(\alpha\)-MC. Secondary BS include UDC, UDCA, UDC, HDCA, and \(\alpha\)-MC. BS content in enteric compartments was quantified by HPLC analysis. Data are presented as median and interquartile range, \(n = 6–7\) per group. Data of PEG-treated rats were compared with those of control rats by Mann Whitney U-tests. \(*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.001\).
species richness, as estimated by Chao1 index was 1,573 ± 209 and 1,639 ± 135 OTUs for control and PEG-fed rats, respectively, showed no significant difference between both groups. Overall, microbiota analysis indicated a trend toward reduced number of fecal bacteria, with no change of richness, but changes in microbiota composition with a relative increase of mucus-associated bacteria in PEG-treated rats.

**DISCUSSION**

Our study shows that PEG treatment changes the microbiota composition and decreases intestinal metabolism of BS and cholesterol in rats. PEG increases the pool size of the primary BS cholate and decreases its fractional turnover rate. Finally, PEG treatment decreases the cytotoxicity of fecal water.

We have previously reported that PEG accelerates whole gut transit time (WGTT) and small intestinal transit in our rats (10). PEG treatment in rats did not induce overt diarrhea because fecal pellets were well formed. WGTT did not correlate with cholate pool size in our study (data not shown). Marcus and Heaton have shown significant correlations between acceleration of WGTT with senna (by 62%) and decreased DC pool (30%) and between prolongation of WGTT with loperamide (by 115%) and increased DC pool (43%) (24). Veysey et al. (39) have also found significant correlations between prolonged colonic transit and increased DC pool size in humans. Serum DC percentage was related to microbial BS-converting enzyme activity and colonic pH (35, 36). It was found that cholate pool size was reciprocally decreased during prolonged colonic transit (39). Berr et al. (4) found that increased activity of microbial BS-converting enzymes in patients with cholesterol gallstones enhances DC pool size in the absence of changes in WGTT (4). Together, the data show that changes in the pool size of primary and secondary BS may be due to alterations in WGTT but could also be facilitated via other mechanisms.

PEG increased the amount of cholate in the cecum contents of rats. Theoretically, this could be caused by decreased ileal cholate absorption, increased intestinal fluid at a stable cholate concentration, or decreased bacterial conversion of cholate. The reabsorption of cholate in quantitative terms did not differ between PEG-treated and control rats. It seems likely that the increased cholate pool size is partly attributable to an increased

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**Fig. 4. BS, neutral sterols (NS), and short-chain fatty acids (SCFA). Distribution of primary (A) and secondary (B) BS in intestinal lumen. SIP, small intestine proximal third; SIM, small intestine middle third; SID, small intestine distal third; Cec, cecum; Col, colon. Secretion of individual BS (C) and primary vs. secondary BS (D) in bile. Primary BS include CDC, C, alloC, α- and β-MC, and 823βMC. Secondary BS include UDC, DC, HDC, and αMC. Fecal NS excretion; Copr, coprostanol; Chol, cholesterol; DIH-Chol, dihydrocholesterol. E: fecal SCFA concentration. F: A, acetate; P, propionate; B, butyrate; L, lactate.**

**Fig. 5. Cytotoxic activity of fecal water as determined by potassium (K) release of human erythrocytes after incubation with fecal water. Data are presented as means ± SD, n = 6–7 per group. Data of PEG-treated rats were compared with those of control rats by unpaired Student’s t-test. **P < 0.01.
volume of luminal contents rather than to increased BS concentrations per volume unit. We observed that PEG largely increased the (watery) content of the intestine, particularly the cecum (97%, \(P < 0.01\)). This large luminal content could have functioned as a “dilution pool” of BS entering the intestine. We hypothesize that, initially, before a new steady state was reached, the dilution of BS due to extra water attracted by PEG in the intestinal lumen would have resulted in decreased BS transport (reabsorption) rate via the apical sodium-dependent BS transporter (Asbt) in the terminal ileum and thus, by negative feedback, resulted in increased BS synthesis. At the level that the dilution pool was “saturated” again to pretreatment levels, both the absolute amount of BS reabsorbed and thereby the synthesis would return to pretreatment levels. The

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Fig. 6. Microbiota. A: quantification of total bacteria in control and PEG-fed rats, expressed as log10 number of 16S copies/g wet feces. Data of PEG-treated rats were compared with those of control rats by Mann Whitney U-tests. B: relative abundance (% of sequences) of bacterial phyla detected in control and PEG-fed rats. C: redundancy analysis on relative abundance of bacteria taxa (genus level) in control (●) and PEG-fed rats (○). D: relative abundance of major bacterial taxa (>5%) that were significantly different between control and PEG-fed rats. E: relative abundance of bacterial taxa that were significantly different between control and PEG-fed rats and that account for <5% of the total sequences. Data of PEG-treated rats were compared with those of control rats by Mann Whitney U-tests. * \(P < 0.05\), ** \(P < 0.01\).
data do not provide strong arguments in favor of an important biological significance of increased cholate in cecum. The stable isotope dilution technique measures the cholate pool size and not the total BS pool size. Although our data can thus not directly be extrapolated toward an increased total BS pool size, the unchanged Cyp7a1 activity seems to indicate that total BS synthesis is also unchanged. Our data furthermore indicate decreased bacterial dehydroxylation of primary BS to secondary BS. In control rats, we found a sharp decrease in cholate in cecum contents compared with small intestinal contents and a concomitant increase in DC. These effects are expected because most of the cholate will be actively absorbed in the distal small intestine (9), and the part that is not absorbed will be mostly converted to DC by the microbiota of the cecum (31). However, in PEG-treated rats, cholate remained higher in the cecum compared with controls and DC appeared minimally (data not shown). Together, these findings indicate decreased microbial dehydroxylation of primary BS in the cecum during PEG treatment.

The first step in intestinal BS metabolism involves deconjugation. Many bacterial species (in particular the predominant Bacteroides) are able to deconjugate BS (25, 31). Free (i.e., unconjugated) primary BS can be further metabolized (for example by dehydroxylation), producing secondary BS. This process seems to be restricted to relatively small populations of gram-positive anaerobes in rodents (25) as well as in humans (25, 31). Narushima et al. (25) showed in germ-free mice inoculated with human flora that all five strains that could be isolated with specific anaerobic culture methods (and that were able to convert cholate to DC) showed the greatest similarity with Clostridium Hylemonae. 7α/β-Dehydroxylation is a multistep pathway that requires the activity of the gene products of the bile acid inducible (bai) genes that are located to a bai operon identified in several Clostridia species (19, 29–31, 40). We observed a decrease in Clostridia during PEG treatment in rats. Although the use of culture-independent analysis of microbiota (pyrosequencing) allowed us to identify a large number of microbes, it did not allow differentiation at the species level. Moreover, to our knowledge, all studies on Clostridia and bai gene products have been performed using human, and not rodent, fecal samples and bacteria. At this point, we cannot directly link decreased sterol conversion during PEG treatment to decreases in specific bai operon-carrying microbiota. PEG may not decrease intestinal sterol conversion by simply reducing the number of specific microbes but could also, rather aspecifically reduce activity of sterol-converting enzymes.

Similar to our results on BS excretion during laxative treatment, Andrieux et al. (1) previously showed that laxative treatment with lactulose in rats decreased fecal hyodeoxycholate and DC and increased 2α-muricholate concentrations. Their results were partly attributed to decreased pH by lactulose but were partly pH independent. The exact mechanism inducing microbial changes during PEG treatment is presently unknown but may include increased intestinal water content and changes in luminal pH. The cytotoxic effect of fecal water is primarily enhanced by BS and fatty acids (28). Fatty acids are not metabolized in the intestinal lumen, and we previously showed that the absorption and excretion of fatty acids is not changed by PEG (37), implying that fatty acids would not differentially influence fecal water cytotoxicity in PEG-treated rats. It should be realized that fecal water cytotoxicity has not been conclusively validated as a biomarker for colon cancer risk. The available literature data consistently show the relationship between changes in BS (profile) and the fecal water cytotoxic activity (20, 28). Studies in the 1990s showed that fecal water cytotoxic activity and colonic cell proliferation were highly correlated in rats (21). Finally, increased colonic proliferation has been considered an early biomarker of increased colon cancer susceptibility (23). Previous studies reported decreased tumor development during PEG treatment in different mouse and rat models of colon cancer (8, 12, 32). A population-based study in patients undergoing colonoscopy showed a lower prevalence of colorectal tumors in PEG4000 users (13). It is therefore tempting to speculate that PEG positively influences gut health by decreasing the production of very hydrophobic DC in the colon, but we cannot exclude that reduced cytotoxicity was unrelated to the secondary BS concentrations. Other factors involved may include a relative increase of mucus-associated bacteria (Akermansia spp.; Ref. 2), shown to be important for a healthy mucus layer in the human gut with respect to mucus production and thickness. Given the frequent and long-term prescriptions of PEG in clinical practice, we believe that human studies are warranted to further delineate the effect of PEG on intestinal microbiota and on BS metabolism.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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