Prebiotic oligosaccharides and the enterohepatic circulation of bile salts in rats

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Van Meer H, Boehm G, Stellaard F, Vriesema A, Knol J, Havinga R, Sauer PJ, Verkade HJ. Prebiotic oligosaccharides and the enterohepatic circulation of bile salts in rats. Am J Physiol Gastrointest Liver Physiol 294: G540–G547, 2008. First published December 13, 2007; doi:10.1152/ajpgi.00396.2007.—Human milk contains prebiotic oligosaccharides, which stimulate the growth of intestinal bifidobacteria and lactobacilli. It is unclear whether the prebiotic capacity of human milk contributes to the larger bile salt pool size and the more efficient fat absorption in infants fed human milk compared with formula. We determined the effect of prebiotic oligosaccharides on bile salt metabolism in rats. Rats were fed a control diet or an isocaloric diet containing a mixture of galactooligosaccharides (GOS), long-chain fructooligosaccharides (lcFOS), and acidified oligosaccharides (AOS) for 3 wk. We determined synthesis rate, pool size, and fractional turnover rate (FTR) of the primary bile salt cholate by using stable isotope dilution methodology. We quantified bile flow and biliary bile salt secretion rates through bile cannulation. Prebiotic intervention resulted in significant changes in fecal and colonic flora: the proportion of lactobacilli increased 344% (P < 0.01) in colon content and 139% (P < 0.01) in feces compared with the control group. The number of bifidobacteria also increased 366% (P < 0.01) in colon content and 282% in feces after the prebiotic treatment. Furthermore, pH in both colon and feces decreased significantly with 1.0 and 0.5 pH point, respectively. However, despite this alteration of intestinal bacterial flora, no significant effect on relevant parameters of bile salt metabolism and cholate kinetics was found. The present data in rats do not support the hypothesis that prebiotics naturally present in human milk contribute to a larger bile salt pool size or altered bile salt pool kinetics.

bile salt kinetics; infant nutrition; cholate

The enterohepatic circulation of bile salts, the major constituents of bile, serves two important functions in the human body. Bile salts enhance the absorption of long-chain saturated fatty acids and fat-soluble vitamins from the intestine. Furthermore, the enterohepatic circulation of bile salts promotes the excretion of lipophilic molecules via the bile into the feces (e.g., cholesterol and bilirubin) and is critically important for cholesterol homeostasis in the body. The dietary fat intake in infants is relatively high compared with the dietary fat intake in adults, whereas the bile salt pool size of the former is lower (33). Efficient absorption of dietary fat is essential for optimal growth and development during infancy.

Human milk is the gold standard in infant feeding. One of the beneficial qualities of human milk involves the more efficient absorption of dietary fat compared with that from formula (34). Interestingly, the bile salt pool is larger in premature infants fed human milk compared with formula, but it has not been clarified whether this contributes to the mechanism underlying the more efficient fat absorption (34). Previously, we reported that the maturation of fat absorption in human neonates is functionally related to an increased capacity to absorb long-chain fatty acids from the intestine, possibly due to developmental changes in bile salt composition and bile salt pool (28).

Prebiotics are nondigestible food ingredients that stimulate the growth and activity of specific bacteria in the colon (i.e., bifidobacteria and lactobacilli) (14). Oligosaccharides are a major constituent of human milk and have been demonstrated to increase the proportion of bifidobacteria and lactobacilli in the infant’s colon (4, 9). The prebiotic galactooligosaccharides (GOS) and fructooligosaccharides (FOS) are known to increase the number of bifidobacteria and lactobacilli in the gut in both human and animal studies (3, 26, 32). Previous studies in rats, using the prebiotic substrate FOS, have shown an increase in the amount of intestinal bifidobacteria and lactobacillus (25, 31).

It is not known whether oligosaccharides, the prebiotic constituent of human milk, influence the bile salt metabolism and thereby play a role in the more efficient fat absorption of breast milk. Interactions between intestinal flora and bile salts are well known. The bacterial metabolism of bile salts in the intestinal lumen can involve deconjugation and dehydroxylation, converting primary bile salts such as cholate and chenodeoxycholate into secondary bile salts (i.e., deoxycholate and lithocholate). Bacterial metabolism of bile salts is partly responsible for the fractional turnover rate (FTR) of primary bile salts, i.e., the portion of the pool that is newly synthesized per day. Bacterial metabolism may also influence the physiological activity of bile salts since secondary bile salts are more hydrophobic than primary bile salts and therefore have a greater capacity to interact with dietary fat.

Recently we developed a method to quantify cholate fluxes in the enterohepatic circulation of experimental animals (19).
This method is based on a stable isotope dilution methodology used in humans that was successfully downscaled to allow measurement of cholate fluxes in experimental animals (19). The enterohepatic circulation of bile salts can be quantitatively characterized by specific kinetic parameters: the pool size (the amount of bile salts in the body), the FTR (the fraction of the pool that is newly synthesized each day), the synthesis rate, and, finally, the cycling time (the time it takes the cholate pool to undergo one full cycle in the enterohepatic circulation). In the present study, we determined the effect of dietary prebiotic oligosaccharides on the enterohepatic circulation of cholate, the major primary bile salt in rats.

**MATERIALS AND METHODS**

*Animals and materials.* Six-week-old male rats (Harlan Laboratories, Zeist, The Netherlands) were housed in a light- and temperature-controlled facility with free access to tap water and to either the prebiotic diet or the control diet. Experimental protocols were approved by the Ethical Committee for Animal Experiments, Faculty of Medical Sciences (University Medical Center Groningen, The Netherlands). 2,2,4,4-Tetradeuterated cholic acid ([2H₄]-CA; isotopic purity, 98%) was obtained from Isotec (Miamisburg, OH). All other chemicals and solvents used were of the highest purity commercially available. Animals received semi-purified AIN-93-based diets pressed (9:1), and 8.8 g acidified oligosaccharides (AOS).

**Plasma and tissue samples.** Blood samples (300 μl) were collected by tail bleeding under isoflurane anesthesia. Blood samples were taken randomly assigned to the prebiotic or the control diet for another 3 wk (n = 8 per group). Body weight was measured weekly; food intake and fecal production were measured during 72 h in the third week after randomization. Three weeks after randomization, relevant parameters of synthesis and enterohepatic circulation of cholate were determined by using the previously mentioned stable isotope dilution technique (19). In short, 3.0 mg of [2H₄]-CA in a solution of 0.5% NaHCO₃ in phosphate-buffered saline was slowly injected via the exchange pool that is newly synthesized each day), the synthesis rate, and, finally, the cycling time (the time it takes the cholate pool to undergo one full cycle in the enterohepatic circulation). In the present study, we determined the effect of dietary prebiotic oligosaccharides on the enterohepatic circulation of cholate, the major primary bile salt in rats.

**Analytical procedures.** Plasma alanine transaminase, aspartate transaminase, alkaline phosphatase, cholesterol, and triglyceride concentrations were determined by routine laboratory techniques. Concentrations of biliary cholesterol and phospholipids were measured as described (13, 16), as were bile salt concentrations in feces and in bile (24). Fecal bile salt composition and fecal neutral sterols were analyzed as follows: 50 mg of dried feces were boiled in 1 ml of alkaline methanol (1 M NaOH-methanol, 1:3 vol/vol) at 80°C for 2 h after addition of 50 nmol 5α-cholestan e and 14 nmol 7α,12α-dihydroxy-5β-cholestan e as internal standard for neutral sterols and bile salts, respectively. After cooling down to room temperature, neutral sterols were extracted by using 3 × 3 ml of petroleum ether, boiling range 60–80°C. The residual sample was diluted 1:9 with distilled water. A sample (100 μl) of the solution was subjected to an enzymatic total bile salt measurement (24). The remaining solution was used for bile salt isolation by reversed-phase solid-phase (C18) extraction (24, 30). The eluate was evaporated to dryness, and bile salts were derivatized to the methyl ester-trimethylsilyl derivatives for gas chromatography analysis. The extracted neutral sterols were derivatized to the trimethylsilyl derivatives by applying the same procedure that was used for bile salts. Bile salt composition of prepared bile samples, fecal samples, and neutral sterol composition of prepared feces samples were determined by capillary gas chromatography on an Agilent gas chromatograph (HP 6890), equipped with a 25 m × 0.25 mm CP-Sil-19-fused silica column (Varian, Middelburg, The Netherlands) and a flame ionization detector. The conditions were as follows: injector temperature 280°C; pressure 16.0 psi; column flow constant at 0.8 ml/min; oven temperature program: 240°C (4 min), 10°C/min to 280°C (27 min); detector temperature 300°C. Hepatic concentrations of triglycerides and cholesterol were measured by using commercial kits (Wako Chemicals, Neuss, Germany and Roche Diagnostics, Mannheim, Germany) after livers were homogenized. Pooled plasma samples were used for lipoprotein separation by fast-protein liquid chromatography (FPLC) on a Superose 6B 10/30 column (Amersham Biosciences). The concentration of triglycerides in the various fractions was determined by using a commercial kit (Wako Chemicals).

**Gas-liquid chromatography electron capture negative chemical ionization mass spectrometry and calculations.** Plasma samples were prepared for isotopic analysis of bile salts by gas chromatography-mass spectrometry (GC-MS) as described (19, 27). Analyses were performed at the pentfluoro-TMS derivative by using a Finnigan SQU7000 Quadrupole GC-MS (Finnigan MAT, San José, CA). GC separation was performed on a 15 m × 0.25 mm column, 0.25-μm film thickness (AT-5MS; Altech Associates, Deerfield, IL). The area ratio M4/M0 is calculated after computerized integration of peak areas of prepared feces samples, fecal samples, and neutral sterol composition by the formula: pool size by the biliary secretion rate of cholate (which was calculated of prepared bile samples, fecal samples, and neutral sterol composition of prepared feces samples were determined by capillary gas chromatography on an Agilent gas chromatograph (HP 6890), equipped with a 25 m × 0.25 mm CP-Sil-19-fused silica column (Varian, Middelburg, The Netherlands) and a flame ionization detector. The conditions were as follows: injector temperature 280°C; pressure 16.0 psi; column flow constant at 0.8 ml/min; oven temperature program: 240°C (4 min), 10°C/min to 280°C (27 min); detector temperature 300°C. Hepatic concentrations of triglycerides and cholesterol were measured by using commercial kits (Wako Chemicals, Neuss, Germany and Roche Diagnostics, Mannheim, Germany) after livers were homogenized. Pooled plasma samples were used for lipoprotein separation by fast-protein liquid chromatography (FPLC) on a Superose 6B 10/30 column (Amersham Biosciences). The concentration of triglycerides in the various fractions was determined by using a commercial kit (Wako Chemicals).

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**Enterohepatic cycling time.** The cycling time of the enterohepatic circulation is the time it takes for a bile salt to undergo one full cycle. The cycling time for cholate can be estimated by dividing the cholate pool size by the biliary secretion rate of cholate (which was calculated by using bile salt composition in bile and bile flow rates after bile cannulation). The fraction of cholate lost per cycle was calculated by...
dividing fractional cholate synthesis rate by cholate cycling frequency.

**Fecal flora composition.** Frozen fecal or colon samples were thawed by adding 1 ml of MilliQ water and heating at 90°C. The suspensions were subsequently homogenized and frozen overnight at −20°C. The homogenized samples were thawed at room temperature, followed by DNA isolation by using the NucliSense Isolation Extraction Kit (BioMerieux, Boxtel, The Netherlands). For the relative quantification of the genera *Bifidobacterium* and *Lactobacillus* in relation to the total bacterial load, a duplex 5′ nuclease quantitative real-time PCR assay was used (15).

Briefly, different primers and probes for the genus *Bifidobacterium* or *Lactobacillus* in combination with primers and probes for total bacteria were used in a temperature profile consisting of 2 min at 50°C, 10 min at 95°C, followed by 45 cycles of 15 s at 95°C and 60°C for 1 min, run on ABI Prism 7700 PCR equipment (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). The relative amounts of the genus *Bifidobacterium* or *Lactobacillus* in the samples were calculated with respect to the total bacterial load according to Liu et al. (23) and expressed in percentages. All samples were analyzed in triplicate.

**Statistics.** Values represent means ± SD for the indicated number of animals per group. Differences between the two groups were determined by Student’s *t*-test for normally distributed values, and Mann-Whitney exact two-tailed *U*-test was used for nonnormally distributed data. *P* < 0.05 was considered significant. Analysis was performed using SPSS 12.0 for Windows software (SPSS, Chicago, IL).

**RESULTS**

**Animal characteristics.** Animals fed the prebiotic diet or the control diet were comparable in body weight, growth, fecal production, and food intake (Table 2). The prebiotic diet significantly elevated serum lathosterol, an intermediate in the cholesterol synthesis pathway, whereas plasma concentrations of cholesterol, triglycerides, alanine transaminase, aspartate transaminase, and alkaline phosphatase were not altered upon treatment (Table 2).

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<th>Table 2. Animal characteristics, plasma and hepatic parameters of lipid metabolism, and liver function after feeding rats a prebiotic or a control diet</th>
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<td><strong>Animal characteristics</strong></td>
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<tr>
<td>Body weight 252 ± 12</td>
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<td>Body weight at termination 456 ± 35</td>
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<td>Feces (wet), g·day⁻¹·100 g body wt⁻¹ 0.4 ± 0.1</td>
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<td>Food intake, g/24 h 27.9 ± 1.7</td>
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<td><strong>Hepatic parameters</strong></td>
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<td>Liver weight -absolute, g 13.8 ± 1.4</td>
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<tr>
<td>Liver weight -relative, % of body wt 3.0 ± 0.2</td>
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<tr>
<td>Triglycerides, nmol/mg liver 14.3 ± 5.4</td>
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<tr>
<td>Cholesterol, nmol/mg liver 5.4 ± 0.7</td>
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<td><strong>Plasma parameters</strong></td>
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<tr>
<td>Alanine transaminase, units/l 46 ± 10</td>
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<td>Aspartate transaminase, units/l 153 ± 75</td>
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<td>Alkaline phosphatase, units/l 6 ± 7</td>
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<tr>
<td>Cholesterol, mmol/l 1.8 ± 0.3</td>
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<tr>
<td>Triglycerides, mmol/l 1.4 ± 0.5</td>
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<td>Lathosterol, μmol/l 0.7 ± 0.1</td>
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**Effect of prebiotic treatment on the composition of intestinal flora.** Figure 1 shows that the prebiotic diet had a prominent bifidogenic effect. After 3 wk the relative numbers of bifidobacteria and of lactobacilli were significantly higher in
colon content and in feces in rats fed the prebiotic diet compared with controls. The proportion of lactobacilli increased 344% ($P < 0.01$) in colon content and 139% ($P < 0.05$) in feces compared with rats on control diet (Fig. 1).

Prebiotic treatment also increased the contribution of bifidobacteria 366% ($P < 0.01$) in colon content and 282% ($P < 0.01$) in feces compared with the control group (Fig. 1). The prebiotic diet significantly lowered pH in both colon content and feces with 1.0 and 0.5 pH point, respectively (Fig. 1).

**Effect of prebiotic intervention on parameters of the enterohepatic circulation of bile salts.** Bile flow and biliary secretion rates of bile salts, phospholipids, and cholesterol were similar between the groups (Fig. 2), as were biliary bile salt composition (Fig. 3), fecal neutral sterol composition, and fecal bile salt excretion (Fig. 4). Prebiotic treatment did not affect the enterohepatic circulation of bile salts. Kinetic parameters of cholate, the main bile salt in rodents, were not significantly altered by the prebiotic diet. Cholate synthesis rate, pool size, and FTR, as well as fecal excretion, were similar in both groups (Table 3 and Fig. 5).

**Effect of prebiotic treatment on hepatic and plasma lipids.** The prebiotic treatment did not affect hepatic and plasma concentrations of cholesterol and triglycerides (Table 2). Upon FPLC separation of plasma lipoproteins, the distribution of triglycerides in the different fractions was comparable in prebiotic-treated rats and controls (Fig. 6).

**DISCUSSION**

We investigated whether prebiotic treatment and subsequent alteration of the intestinal bacterial flora affects the enterohepatic circulation and composition of bile salts in rats. The results show that prebiotic treatment significantly increased the relative contributions of both lactobacilli and bifidobacteria in the colon and the feces of rats. Furthermore, pH in both colon and feces significantly decreased after feeding rats a prebiotic diet. This prebiotic effect, however, did not significantly affect bile flow or bile salt composition or the synthesis and enterohepatic circulation of cholate.

The liver parenchymal cells synthesize the primary bile salts cholate and chenodeoxycholate. Primary bile salts are conjugated before their secretion into bile (in rats predominantly to taurine). Upon entering the proximal small intestine, conjugated bile salts stimulate the emulsification and absorption of dietary fat. Conjugated bile salts are efficiently reabsorbed in the terminal ileum, mediated by the apical sodium-dependent bile salt transporter (Asbt) and the basolateral transporter Ost $\alpha/\beta$. Primary bile salts that escape absorption in the terminal
Values are expressed in means ± SD of n = 7 or 8 per group. No significant differences between prebiotic-treated rats compared with controls. C, cholate; DC, deoxycholate; CDC, chenodeoxycholate; α-M, α-muricholate; β-M, β-muricholate; Δ22-M, Δ22-muricholate; HDC, hyodeoxycholate.

Fig. 3. Effect of prebiotic treatment (solid bars) on biliary bile salt composition compared with control rats (open bars). Data are expressed in means ± SD of n = 7 or 8 per group. No significant differences between prebiotic-treated rats compared with controls. C, cholate; DC, deoxycholate; CDC, chenodeoxycholate; α-M, α-muricholate; β-M, β-muricholate; Δ22-M, Δ22-muricholate; HDC, hyodeoxycholate.

Fig. 4. Effect of prebiotic treatment on fecal bile salt excretion (A) and fecal neutral sterol excretion (B). Prebiotic diet (solid bars), control diet (open bars). Values are expressed in means ± SD, and n = 8 per group. No significant differences between prebiotic-treated animals compared with controls in any of the parameters. Copr, coprostanol; epiCopr, epicoprostanol; Chol, cholesterol; Dih-Chol, dihydrocholesterol.

Table 3. Pool size, fractional turnover rate, and synthesis rates of cholate

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<th>Prebiotic Diet</th>
<th>Control Diet</th>
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<tr>
<td>Fractional turnover rate, day⁻¹</td>
<td>0.28±0.04</td>
<td>0.26±0.07</td>
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<tr>
<td>Pool size, μmol/100 g body wt</td>
<td>8.11±1.82</td>
<td>8.80±2.57</td>
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<tr>
<td>Synthesis rate, μmol·100 g⁻¹·day⁻¹</td>
<td>2.25±0.55</td>
<td>2.23±0.72</td>
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Values are obtained by [2H₄]-cholic acid isotope enrichment measurements in plasma of rats fed the prebiotic diet or the control diet, as detailed in MATERIALS AND METHODS.

The present prebiotic treatment resulted in lactobacilli, a decrease in fecal pH, and a significant increase of fecal bifidobacteria of almost 60% in healthy infants (20). Vos et al. (32) used an identical prebiotic diet (10 wt% GOS-FOS; ratio 9:1) for 6 wk, which resulted in an increase of fecal bifidobacteria of almost 60% in healthy infants (20). The addition of a GOS-POS mixture to infant formula resulted in an increase in fecal bifidobacteria of 40% in healthy infants (20). Vos et al. (32) used an identical prebiotic diet (10 wt% GOS-FOS; ratio 9:1) in a murine model, resulting in 40% bifidobacteria of total bacterial load in fecal samples.
Surprisingly, the present study showed a contribution of bifidobacteria of 1.3 and 1.5% in feces and colon content, respectively, after prebiotic treatment. Thus, although the presently used diet resulted in an increase of lactobacillus in the same range as mice and humans and a significant decrease in fecal and colonic pH, the less pronounced bifidogenic response could theoretically contribute to the observed lack of effect on the enterohepatic circulation of bile salts.

It should be realized that the situation studied in the present rat model differs in several aspects from the physiology in human infants. Besides the less pronounced prebiotic response, it cannot be excluded that the absence of a gallbladder in rats influences the dynamics of the enterohepatic circulation of bile salts. Furthermore, immaturity of intestinal absorption of nutrients (28) and bile salts (6) intervene with the enterohepatic circulation of bile salts, which may render the dynamics of bile salt metabolism in human infants not completely comparable to the presently studied rat model. Finally, only in rats, the primary bile salt chenodeoxycholate is further metabolized to α-muricholic acid and β-muricholic acid, rendering the bile salt pool more hydrophilic compared with the human situation. In the present experiment, however, the amount of muricholic bile salts measured in bile did not exceed 25%. Nevertheless, we are aware of the possibility that one or more of the aforementioned differences may limit the extrapolation of the present results to the human situation.

Besides the addressed differences in bile salt physiology between human infants and the presently used rat model, human milk contains high concentrations of cholesterol (2.6–3.9 mmol/l) compared with formulas (0.3–0.9 mmol/l) (5). The colonic flora of human infants develops its dehydroxylating capacity over the first months of life (2), whereas in the adult rat the dehydroxylating bacteria are well established. It is tempting to speculate that the larger bile salt pool size found in breast-fed infants could be related to the substantially higher dietary intake of cholesterol and not, based on our present observations, on differences in dietary oligosaccharides. Enhanced conjugation of bile salts by bile hydrolase-producing bacteria such as lactobacilli could, in theory, be expected to increase the amount of deconjugated bile salts and the subsequent fecal excretion hereof. In the present study, both amount and composition of fecal bile salts were unaffected upon prebiotic treatment. We hypothesize that this can be due to the relative low contribution of lactobacillus and bifidobacteria to the total intestinal deconjugating capacity. Bile salt hydrolase is absent from lactobacillus and bifidobacteria, also detected in other bacterial species (12). Furthermore, efficient passive absorption of deconjugated bile salts may have masked a deconjugating, enhancing effect of prebiotic treatment.

The presently applied methodology allowed determination of bile salt kinetic parameters in a physiologically uncompromised animal model. The parameters of cholate kinetics determined in this experiment were in line with previous experiments performed in rats by using the same method (19). Under steady-state conditions, the bile salt pool size is regulated by hepatic de novo synthesis of bile salts and by the efficiency by
which bile salts are reabsorbed in the intestine. Under steady-
state conditions, the amount lost via the feces is equal to the
amount of bile salts newly synthesized by the liver. A differ-
ence neither in cholate synthesis rate nor in fecal bile salt
excretion was observed, indicating that prebiotic treatment
does not affect the two important rate-limiting parameters of
the enterohepatic circulation. We also observed that the fecal
excretion of chenodeoxycholate-derived metabolites (mur-
icholate and hyodeoxycholate) was not altered either, suggest-
ing no effect of prebiotic treatment on the synthesis of the oth-
er primary bile salt, chenodeoxycholate.

Apart from an important role in absorption of dietary lipids,
bile salts are critically involved in cholesterol homeostasis in
the body. Prebiotic substances are suggested to have a plasma
lipid-lowering effect in both animal and human studies. Serum
cholesterol concentrations in bottle-fed infants decreased as the
number of lactobacilli in their stools increased (18). Possible
mechanisms could be modulation of the intestinal bacterial
flora with production of short-chain fatty acids inhibiting
hepatic fatty acid or cholesterol synthesis or alteration of the
bacterial bile acid deconjugating capacity and a subsequent
altered fecal bile salt excretion (8). Studies in rats have con-
sistently shown a decrease in plasma triglycerides after a diet
supplemented with inulin and oligofructose (reviewed in Ref.
7). Data on plasma cholesterol, however, are less straightfor-
ward. A 10 wt% oligofructose-supplemented diet decreased
serum cholesterol concentrations in rats in one study (11), but
the addition of oligofructose to the diet of lean rats did not
induce a decrease in plasma cholesterol levels in another study
(21). Human studies investigating the effect of oligofructans on
lipid metabolism have shown variable results (reviewed in Ref.
8). Our prebiotic diet did not significantly affect plasma con-
centrations of cholesterol in rats, whereas it increased the
serum lathosterol concentration. Lathosterol is an intermediate
in the cholesterol synthesis pathway, which could suggest that
cholesterol synthesis was enhanced during prebiotic treatment.
However, cholesterol synthesis was not determined in this
experiment, and no other indications of increased cholesterol
synthesis were obtained. For example, the hepatic mRNA
expression of HMG-CoA reductase, encoding for the rate-
limiting enzyme of cholesterol synthesis, was not upregulated
by prebiotic treatment (data not shown). The presently used
diet did not affect serum triglycerides or hepatic triglycerides.
Also, the amount of triglycerides in the VLDL fraction of
lipoproteins was comparable in prebiotic-treated and control
rats, indicating that the present treatment did not affect hepatic
VLDL composition or triglyceride distribution over the various
plasma lipoproteins (Fig. 6). Rat studies on lipid metabolism or
bile salt metabolism using diets supplemented with the pres-
ently used GOS-IcFOS, have, to the best of our knowledge, not
been performed. In accordance with the present results, a study
recently performed in human infants showed unaffected
plasma levels of cholesterol and of triglycerides in infants fed
a formula supplemented with identical prebiotic substances in
the same ratio used in the present study (1).

In summary, we conclude that feeding rats a prebiotic diet
induces modification of the intestinal flora and decreases the
intestinal pH in the colon and the feces. However, the prebiotic
diet does not influence the metabolism of bile salts in rats. The
present data in rats do not support the hypothesis that prebiotics

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