Bile acids are natural detergents involved in cholesterol homeostasis. These acids are formed from cholesterol in the liver, and their synthesis represents an important pathway for the elimination of cholesterol from the body.1 The bile acid biosynthetic pathway requires a number of enzymatic modifications of the cholesterol backbone, followed by β-oxidation of the cholesterol side chain.1 In humans, the bile acid concentration is represented mainly by the primary acids cholic acid (CA) and chenodeoxycholic acid (CDCA) (reviewed by Monte et al.2). These acids are conjugated with taurine and glycine to form amidated detergents. After their delivery into the intestine, primary bile acids are subjected to dehydroxylation by bacterial 7α-dehydroxylase in the gastrointestinal tract. This reaction converts CDCA and CA into the secondary acids lithocholic acid (LCA) and deoxycholic (DCA) (−22.3%), and hyodeoxycholic (HDCA) (−19.2%) acids. A gender-related difference was observed in the responses of various bile acids, and the total bile acid concentration was significantly reduced only in men (−18.6%), whereas it remained almost unchanged in women (+0.36%). This difference suggests that fenofibrate would be more efficient at reducing bile acid toxicity in men than in women in cholestatic liver diseases.

Fenofibrate belongs to the group of hypolipidemic fibrates that act as activators of the peroxisome proliferator–activated receptor-α (PPARα), which is a regulator of bile acid synthesis, metabolism, and transport. The present study aimed at evaluating the effects of fenofibrate on the circulating bile acid profile in humans. A study population of 200 healthy individuals comprising both genders completed a 3-week intervention with fenofibrate, and 17 bile acid species were measured in serum samples drawn before and after fenofibrate treatment. Fenofibrate caused significant reductions in levels of chenodeoxycholic (CDCA) (−26.4%), ursodeoxycholic (UDCA) (−30.5%), lithocholic (LCA) (−18.4%), deoxycholic (DCA) (−22.3%), and hyodeoxycholic (HDCA) (−19.2%) acids. A gender-related difference was observed in the responses of various bile acids, and the total bile acid concentration was significantly reduced only in men (−18.6%), whereas it remained almost unchanged in women (+0.36%). This difference suggests that fenofibrate would be more efficient at reducing bile acid toxicity in men than in women in cholestatic liver diseases.
various genes that are crucial for triglyceride/cholesterol metabolism and gluconeogenesis.9

Fibrates exert beneficial effects on liver biochemistry in patients with PBC.10–14 These benefits have been associated primarily with the lipoprotein-lowering and anti-inflammatory properties of PPARα activators.10,14 In addition, PPARα is an important regulator of genes that encode bile acid–synthesizing and –metabolizing enzymes such as the cytochrome P450s (CYP)7A1, CYP8B1, and CYP27; UDP-glucuronosyltransferase 2B4 (UGT2B4); and sulfotransferase 2A1 (SULT2A1).6,8,15,16 Various PPARα activators also modulate hepatic and/or intestinal transporters involved in bile acid uptake and/or excretion, such as the Na+-taurocholate cotransporting polypeptide (SLC10A1), the organic anion-transporting polypeptide 1B (SLCO1B1), the biliary salt export pump (ABCB11), the apical sodium-dependent bile acid transporter (SLC10A2), and the organic solute transporters θα and θβ (reviewed in ref. 17). Overall, these observations suggest that pharmacological activators of PPARα such as fenofibrate may also modulate bile acid homeostasis in humans. We sought to test this hypothesis by examining the profile of circulating bile acids in pre- and post-treatment sera from 200 noncholestatic volunteers treated with fenofibrate for 3 weeks.

RESULTS

Analytical method

The method involving liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) allowed the simultaneous determination of 17 bile acid species in a single serum sample. The analytical run required only 15 min (Figure 1a), and the lower limit of detection varied from 2.2 (LCA sulfate, LCA-S) to 8.0 nmol/l taurochenodeoxycholic acid (TCDCCA) (Figure 1b).

Baseline profiles of serum bile acids

The baseline composition of bile acids is illustrated in Table 1. The serum bile acids consisted mainly of conjugated and unconjugated primary acids (62%), whereas secondary acid species were only half as abundant (30%), and the 6α-hydroxylated acids HDCA and HCA together represented only 2.3% of total bile acids. Unconjugated bile acids (1,009.1 ± 74.3 nmol/l) and glycine conjugates (1,238.1 ± 84.4 nmol/l) represented 40 and 49% of the species, respectively. Taurine-conjugated acids (266.7 ± 25.7 nmol/l) were less abundant (10%). Overall, the species distribution in serum bile acid was GDCA≥CDCA>GDCA≥CDCA>GCA≥CA>UDCA=TCA. All other species were present at concentrations <100 nmol/l (Table 1).

Changes in bile acid levels after fenofibrate treatment

Treatment with fenofibrate resulted in significant reductions in the serum concentrations of CDCA (−26.4%, P < 0.001), urso-deoxycholic acid (UDCA) (−30.4%, P < 0.001), LCA (−18.4%, P < 0.001), glycolithocholic acid (GLCA) (−7.8%, P < 0.05), LCA-S (−23.1%, P < 0.01), DCA (−22.3%, P < 0.001), tauro-deoxycholic acid (TDCA) (−27.6%, P < 0.01), and HDCA (−19.2%, P < 0.001), whereas tauroursodeoxycholic acid (+50.5%, P < 0.001) and HCA (+33.5%, P < 0.001) levels were significantly increased and other species were not significantly altered (Table 1). Because 6 of the 10 affected bile acids were unconjugated, the total level of free acids was also significantly reduced (−21.4%, Table 1). Furthermore, the 22.3% reduction in DCA concentration (from 393.0 to 305.6 nmol/l, P < 0.001) entailed a reduction in the total (conjugated and unconjugated) level of this species (i.e., DCA+TDCA+GDCA: −14.4%, P < 0.001) as well as in total secondary acids (unconjugated and conjugated forms of LCA and DCA: −13.9%, P < 0.001, Table 1).

Gender-related differences in baseline and postfenofibrate profiles of serum bile acids

A comparison of the baseline bile acid profiles in men and women revealed several gender-related differences (Table 2). Serum samples from women contained a significantly smaller amount of the primary acids CDCA (P < 0.01) and CA (P < 0.01) and also of the 6α-hydroxylated acid, HCA (P < 0.01). Furthermore, the total levels of CA (P < 0.05), primary acids (P < 0.05), and unconjugated acids (P < 0.05) were also significantly lower in women.

Changes after fenofibrate administration were analyzed separately in men and women, and significant differences were observed between the two groups with respect to bile acid profiles (Figures 2–4). After fenofibrate administration, the total bile acid concentration was significantly reduced in men (−18.6%, P < 0.01), whereas it remained almost unchanged in women (+0.36%) (Figure 2a). The effects of fenofibrate on total
bile acid concentration were significantly stronger in men than in women \((P < 0.05)\). However, the statistical significance of this difference was reduced when the response to treatment was adjusted for body mass index \((P = 0.051)\) and body weight \((P = 0.151)\).

Given the statistically nonsignificant difference between the two gender groups with regard to changes in taurocholic acid (TCA) levels \((-41.7\% \text{ in men and } +63.3\% \text{ in women, Figure 3a})\), the total of taurine conjugates showed a gender-dependent variation, with a 23.2\% reduction in men and a 52.2\% increase in women \((P = 0.051)\). However, the reduction in men failed to reach statistical significance. Taurolithocholic acid (TLCA) levels were not significantly changed in either gender group, whereas taoursodeoxycholic acid and TDCA concentrations were increased in both genders, reaching statistical significance only in women \((Figure 3b,c)\).

As shown in \(Figure 2c\), the level of total glycine conjugates was not significantly affected by fenofibrate in either men or women. This finding reflects the absence of significant changes in glycochenodeoxycholic acid (GCDCA) and glycodeoxycholic acid (GDCA) levels in samples from the two genders \((Figure 3d,e)\). On the other hand, GLCA levels were significantly reduced in men, whereas glycocholic acid (GCA) levels were significantly increased only in women \((Figure 3e,f)\).

The unique gender-related difference observed for unconjugated species specifically affected CA levels, which were

### Table 1 Changes in serum bile acid profiles after a 3-week treatment period with fenofibrate

| Bile acid | Before fenofibrate | After fenofibrate |
|---------|------------------|------------------|
|         | Mean(SEM) | 10th | 90th  | Mean(SEM) | 10th | 90th  |
| CDCa | 256.9(29.5) | 16.4 | 610.9 | 189.2*** | 20.8 | 570.1 |
| TCDca | 92.9(8.8) | 12.7 | 268.5 | 103.1 | 9.5 | 242.3 |
| GCDca | 745.9(54.2) | 169.8 | 1,694.2 | 703.8 | 46.4 | 1,656.3 |
| CA | 176.2(27.1) | 2.6 | 403.2 | 159.7 | 23.0 | 389.7 |
| TCA | 113.7(15.8) | 8.1 | 269.5 | 112.0 | 16.1 | 203.3 |
| GCA | 198.4(16.9) | 29.8 | 461.5 | 227.3 | 18.5 | 531.2 |
| UDCA | 106.2(7.9) | 13.6 | 269.1 | 73.9*** | 5.0 | 166.8 |
| TUDCA | 6.1(1.2) | 0.00 | 13.3 | 9.2*** | 1.0 | 27.7 |
| LCA | 17.6(1.3) | 4.4 | 34.3 | 14.3*** | 1.3 | 3.2 |
| TLCA | 19.1(1.1) | BLQ | 32.3 | 19.9 | 1.1 | 32.3 |
| GLCA | 18.4(1.7) | 4.6 | 35.0 | 16.9* | 1.9 | 35.9 |
| LCA-S | 7.0(0.6) | BLQ | 14.4 | 5.4** | 0.5 | 11.0 |
| DCA | 393.1(24.6) | 102.9 | 782.4 | 305.6*** | 19.4 | 627.0 |
| TDCA | 35.1(3.2) | 4.5 | 81.6 | 44.8** | 3.9 | 104.0 |
| GDCA | 275.5(19.3) | 62.5 | 625.2 | 252.2 | 18.9 | 539.0 |
| HDCA | 53.5(3.0) | 17.4 | 104.5 | 43.2*** | 2.3 | 81.8 |
| HCA | 5.5(0.5) | BLQ | 13.6 | 7.4*** | 0.6 | 17.5 |
| Total CDCa | 1,095.7(76.5) | 257.3 | 2,414.3 | 996.1 | 59.6 | 217.9 |
| Total CA | 488.2(40.4) | 74.6 | 1,245.4 | 499.0 | 38.5 | 82.3 |
| Total DCA | 703.7(40.4) | 201.8 | 1,465.3 | 602.6*** | 36.2 | 1,217.1 |
| Total LCA | 62.0(3.2) | 14.6 | 106.2 | 56.5* | 3.2 | 98.8 |
| Total primary | 1,583.9(109.4) | 354.1 | 3,504.5 | 1,495.1 | 89.4 | 3,312.4 |
| Total secondary | 765.7(42.6) | 241.6 | 1,566.6 | 659.2*** | 38.1 | 1,313.4 |
| Total 6α-hydroxy | 59.0(3.1) | 21.6 | 110.1 | 50.6** | 2.4 | 91.4 |
| Total free | 1,009.1(74.3) | 252.8 | 2,056.6 | 793.3*** | 55.2 | 1,685.2 |
| Total glyco | 1,238.1(84.4) | 318.1 | 2,536.7 | 1,200.3 | 77.1 | 2,551.5 |
| Total tauro | 266.7(25.7) | 35.7 | 747.9 | 288.9 | 27.5 | 606.1 |
| Total | 2,520.9(142.5) | 795.1 | 5,015.2 | 2,287.9 | 116.9 | 4,434.6 |

Bile acid concentrations are expressed in nmol/l. Statistically significant differences in bile acid concentrations were determined by the Wilcoxon matched-pairs signed-ranks test: \(\ast P < 0.05; \ast\ast P < 0.01; \ast\ast\ast P < 0.001\).

aPercentile. bSum of all bile acids.

BLQ, below the limit of quantification; CA, cholic acid; CDCa, chenodeoxycholic acid; DCA, deoxycholic acid; GCA, glycocholic acid; GCDca, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; glyco, glycoconjugates; HCA, hyocholic acid; HDCA, hyodeoxycholic acid; LCA, lithocholic acid; LCA-S, LCa-sulfate; tauro, tauroconjugates; TCA, taurocholic acid; TDCCA, taurochenodeoxycholic acid; TDCa, taurodeoxycholic acid; TLCA, taurlithocholic acid; TUDCa, taursodeoxycholic acid; UDCA, ursodeoxycholic acid.
significantly reduced in men (−28.4%, \( P < 0.05 \)) but were not significantly increased (\( +22.2\% \)) in women (Figure 4a). With the notable exception of serum HCA, which was significantly more abundant in both genders after treatment, unconjugated species (i.e., CDCA, UDCA, LCA, DCA, and HDCA) were reduced in both genders (Figure 4). Likewise, serum levels of LCA-S were reduced in both men and women, but the reduction reached statistical significance only in women (Figure 4b).

Overall, these observations reveal the presence of gender-related differences in circulating levels of bile acids as well as in individual responses to fenofibrate.

**DISCUSSION**

This study provides a comprehensive analysis of the effects of fenofibrate on the profile of circulating bile acids. This
mediated accumulation of SULT2A1 protein in the liver cannot compensate for the decreased availability of its substrate. Another interesting observation is the differential response of CDCA and CA levels. PPARα was previously identified as a positive regulator of the CYP8B1 gene in the human and murine liver. This gene encodes 12α-hydroxylase, a key branch in the bile acid biosynthetic pathway that favors the formation of CA instead of CDCA, thereby determining the CA/CDCA ratio. It is therefore interesting that, in the current study, fenofibrate caused a greater reduction in CDCA levels than in CA levels (Table 1). Such a difference results in an increase of the CA/CDCA ratio from 0.68 in pretreatment samples to 0.84 in post-fenofibrate samples. This increase is gender-dependent because CA levels were reduced in men whereas they underwent significant increases in women. In both genders, CDCA was reduced; in men, the CA/CDCA ratio remained unchanged (0.69 and 0.71 before and after fenofibrate, respectively) whereas in women it increased from 0.67 to 1.01. These observations might indicate that the PPARα-dependent upregulation of CYP8B1 is stronger in women than in men.

The response to fenofibrate almost erases the gender-related difference in baseline bile acid levels discussed above. In fact, fenofibrate entails a “feminization” of the bile acid profile in men. Indeed, the total bile acid concentration in women was greater than in men. Interestingly, previous studies reported similar gender-related differences in the response to fenofibrate. For example, in C57BL/6J mice fed a high-fat diet, fenofibrate reduced gain in body weight and total cholesterol only in male animals. Liu et al. recently reported from the GOLDN (Genetics of Lipid Lowering Drugs and Diet Network) study that fenofibrate differentially affected the concentrations of large low-density lipoprotein particles in men and women. These observations further support the existence of gender specificity in the response to fenofibrate for selected parameters such as bile acid levels.

Nevertheless, a significant number of bile acid parameters are affected in a similar manner in men and women. These
include the total secondary acids as well as the total DCA and total LCA levels; all of these are reduced in all post-treatment samples from both genders, although specific acids (i.e., TLCA and GLCA) may be differentially affected depending on gender (Figure 3). The secondary acids DCA and LCA are hydrophobic molecules and are among the most toxic bile acids.\(^{27,28}\) The reduction in their concentration levels suggests that fenofibrate might contribute to decreasing the toxicity of specific bile acids. Accumulation of toxic bile acids is a hallmark of chronic cholestatic liver diseases such as PBC and PSC.\(^2\) Interestingly, recent pilot studies have reported a positive effect of fenofibrate on the liver functions of patients with PBC resistant to the classic anticholestatic drug UDCA.\(^{10–14,29}\) These benefits were associated primarily with the lipoprotein-lowering and anti-inflammatory properties of PPARα activators. However, results from the present study support the hypothesis that an additional favorable effect of fenofibrate in these patients might be a reduction in the levels of toxic secondary bile acids.

On the other hand, the fact that fenofibrate preferentially reduces bile acid concentrations in men also suggests that the drug may be more efficient at reducing the cytotoxic properties of these detergents in men than in women. Cholestatic diseases such as PBC and PSC are gender-related pathologies; middle-aged women constitute the majority of patients with PBC, whereas PSC affects children, teenagers, and adults, with a predominance among males.\(^7\) Consequently, the data from our study suggest that fenofibrate may be more efficient at correcting bile acid toxicity in male patients with PSC than in women with PBC. In agreement with this, Chazouillères et al. recently reported that combination therapy with fenofibrate and UDCA induces significant biochemical improvements in patients with PSC who have incomplete response to UDCA.\(^{30}\) However, further studies are needed to evaluate the extent of bile acid reduction attained with the use of fenofibrate in these patients and to determine whether such a reduction might contribute to improvement in liver functions.

In conclusion, this study provides evidence that fenofibrate alters the profile of circulating bile acids in humans, an effect that is thought to have a role in the reported improvement in liver functions observed in fenofibrate-treated patients with PBC. Furthermore, the gender-related differences detected suggest that fenofibrate could be more efficient in reducing bile acid toxicity in men than in women.

### METHODS

**Materials.** Unconjugated and taurine- and glycine-conjugated bile acids were purchased from Steraloids (Newport, RI). Deuterated bile acids (d4-Ca, d4-CDCA, d4-LCA, and d4-DCA) were purchased from C/D/N Isotopes (Montreal, Quebec, Canada). Protein assay reagents were obtained from Bio-Rad Laboratories (Marnes-la-Coquette, France). High-performance liquid chromatography-grade solvents were obtained from VWR Canlab (Montreal, Quebec, Canada). Strata-X 33-μm polymeric reversed-phase solid-phase extraction columns (60 mg/3 ml) were obtained from Phenomenex (Torrance, CA).

**Study participants.** The population for this study consisted of 200 Caucasian participants (100 men and 100 women) from the GOLDN study who were recruited exclusively at the University of Minnesota Field Center (Minneapolis, MN). The demographic data and laboratory characteristics of these volunteer participants are shown in **Table 3**.

GOLDN is a single-arm, uncontrolled, nonrandomized intervention aimed at identifying genetic factors associated with interindividual variability in the triglyceride response to high-fat meals and fenofibrate.\(^{31–34}\) The study is part of the Program for Genetic Interaction (PROGENI) and is funded by the National Institutes of Health.\(^{22,31–34}\) Only participants who had not taken lipid-lowering agents for at least 4 weeks before the initial visit were included. As described by Lai et al.,\(^{33}\) the participants made five visits during the course of the study. The serum samples used in our study were drawn at visits 1 and 3. Between these two visits, the participants were given 160 mg of fenofibrate/day (TriCor; Abbott Laboratories, Chicago, IL) and were instructed to take one tablet once a day with breakfast for 3 weeks.\(^{31,34}\) Adherence to fenofibrate therapy was assessed primarily by means of tablet counts. Generally, medication adherence was considered acceptable (using an arbitrary threshold of compliance) when study participants had taken >75% of their expected doses prior to their study day. This threshold was achieved by 99.0% (99/100) of the men and 97.0% (97/100) of the women in this cohort.

At both visits, blood was drawn after a 12-h fast, and serum was isolated and frozen at −80 °C until subsequent analyses. Because this was a study of ambulatory outpatients who were generally healthy, no attempt was made to standardize diet within or between subjects prior to the requisite 12-h fasting baseline and postfenofibrate assessments. Of course, we cannot exclude the possibility of dietary changes during the study, but we would expect that any significant changes in diet would be rare and, in the context of generally healthy individuals, have minimal, if any, impact on the findings of this study.

Exclusion criteria included the following: fasting triglycerides ≥1,500 mg/dl; recent history (within 6 months) of myocardial infarction, coronary bypass surgery, coronary angioplasty, or percutaneous transluminal coronary angioplasty; self-report of a positive history of liver, kidney, pancreas, or gall bladder disease or a history of malabsorption of nutrients; current use of insulin or warfarin; serum concentrations of aspartate aminotransferase >52 U/l in men and >42 U/l in women; serum concentrations of alanine transaminase >66 U/l in men and >44 U/l in women; glomerular filtration rate <30 l/min/1.73 m² estimated from the Modification of Diet in Renal Disease equation; pregnant women and women of childbearing potential not using contraception; and women who were breastfeeding their infants. Individuals who reported current use of prescription and/or over-the-counter hypolipidemic drugs or dietary supplements known to influence lipid values (e.g., fish oil, flaxseed oil, niacin) were required to consult with their physician for approval to discontinue these lipid-lowering agents for 4 weeks prior to study participation. Although known history of liver disease was an exclusion criterion, we cannot exclude the possibility of undiagnosed underlying liver disease. This may be considered a possible limitation of this study.

A description of the comorbidities of subjects included in this cohort resembles that of the overall GOLDN study;\(^{33}\) these comorbidities have limited relevance to liver disease and function. Specifically, 8% of our population had type 2 diabetes mellitus (evenly split between men and women).

### Table 3 Clinical and laboratory characteristics of the volunteers

| Characteristic       | Men (n = 100) | Women (n = 100) | Total (N = 200) |
|----------------------|--------------|----------------|-----------------|
| Age (years)          | 52.42 ± 1.45 | 52.73 ± 1.53   | 52.6 ± 14.9     |
| Alanine aminotransferase (U/L) | 30.5 ± 1.20  | 19.6 ± 0.65    | 25.05 ± 11.05   |
| Aspartate aminotransferase (U/L) | 31.82 ± 0.74 | 26.83 ± 0.56   | 29.33 ± 7.01    |
| Creatinine (mg/dl)   | 0.915 ± 0.015 | 0.736 ± 0.016  | 0.826 ± 0.179   |
| Weight (lb)          | 204.2 ± 2.7  | 165.9 ± 3.6    | 185.1 ± 3.7     |
| Body mass index (kg/m²) | 29.3 ± 0.5  | 28.1 ± 0.6     | 28.7 ± 0.5      |
| Waist (cm)           | 101.9 ± 1.1  | 90.3 ± 1.7     | 96.1 ± 1.5      |
women) and 5% had some form of cardiovascular disease (predomi-
nantly men). In addition, 27% had a diagnosis of hypertension and 44% had symptoms that met the definition of metabolic syndrome (both of these conditions were evenly distributed between the genders). Given the exclusion criteria described above, specifically in regard to measures of liver function (aspartate aminotransferase/alanine transaminase), we believe that there is no obvious systematic basis for presumed effects of comorbidities on our major findings from this study. With respect to coadministered drugs, few subjects were taking any chronic drug therapy (including nutraceuticals and over-the-counter medications) known to affect bile acids. One possible exception may be those who reported receiving chronically administered estrogens or progestins (or a combina-
tion of these). The number of subjects taking any form of systemic estrogen or progestin (or a combination) was limited to 30 of the 100 women participating in this cohort. Based on their declared indication or presumed indication, 15 individuals were using them for hormonal replacement therapy (n = 15) and 15 were using them for contraception. Given that these medications are usually taken on a chronic basis, their impact on the observations of fenofibrate's effect on bile acid disposition would be expected to be modest.

The protocol was approved by the institutional review boards at the University of Minnesota, Laval University, and the CHUQ Research Center. Written informed consent was obtained from all participants.

**Bile acid measurements.** Bile acid concentrations were determined with a high-performance LC–MS/MS system with an electrospray interface, using a novel method adapted from that described by Ye et al. The modified method allows the simultaneous evaluation of 17 species in the same sample (Figure 1a). Solid-phase extraction was initiated by adding 2 ml of a 0.1% (wt/vol) ammonium formate solution and 30 µl of internal standards (i.e., the deuterated bile acids d4-CDCA, d4-CA, d4-LCA, and d4-DCA) to 100 µl of serum. The same treatment was applied to analyti-
cal standards, which were diluted (1:1) with 100 µl of adsorbed serum and subsequently used to generate calibration equations. Solid-phase extraction columns were conditioned with 1 ml MeOH and 2 ml of 0.1% formic acid. The columns were successively washed with 2 ml of H2O and 2 ml of H2O:MeOH (80:20) containing 0.1% formic acid under negative pressure. Bile acids were eluted with 2 ml of MeOH. The eluates were completely evaporated at 45 °C under N2 and reconstituted in 100 µl of H2O:MeOH (50:50) containing 5 mmol/l ammonium acetate and 0.01% formic acid. Fifteen µl of either the sample or calibration standard was then injected into the LC-MS/MS system.

A single LC method was used for the separation of the various con-
jugates of bile acids: free (CDCA, CA, UDCA, LCA, DCA, HDCA, and HCA), taurine (TCDDCA, TCA, taoursodeoxycholic acid, TILCA, and TDC), glycine (GCDDCA, GCA, GLCA, and GDCa), and sulfate (LCA-
S) (Figure 1). The chromatographic system consisted of an Alliance 2690 Separations Module (Waters, Milford, MA). Analytes were separated using a 50 x 3 mm Synchrony Hydro-RP column (2.5-µm particles) (Phe-
omenex, Torrance, CA). The chromatographic conditions used were: 5 mmol/l ammonium acetate–0.01% formic acid in MeOH (solvent B), 5 mmol/l ammonium acetate–0.01% formic acid in MeOH (solvent B), and acetonitrile (solvent C) at a flow rate of 800 µl/min. The chromatographic program was as follows: (i) initial conditions were 40% A:55% B:5% C for 4 min; (ii) a linear gradient to 80% B was applied over the next 8 min; (iii) the column was flushed with 90% B for the next 2 min; and (iv) re-equilibration to the initial conditions took place over the next 4 min. All analytes were quantified by MS/MS using an API3200 LC–MS/MS instrument (Applied Biosystems, Concord, Ontario, Canada). The temperature of the source was set at 350°C, and the parameter settings used are shown in Figure 1b.

**Data analysis.** Baseline characteristics were calculated as mean values and ranges (10th–90th percentiles). The response to treatment was calculated as the difference in bile acid concentrations after treatment as compared to the values before treatment. The total bile acid concen-
tration corresponds to the sum of the 17 bile acid concentrations. The

sums of glyco- and tauroconjugates were calculated by adding the con-
centrations of conjugated CDCA, CA, DCA, and LCA. The sum of free (i.e., unconjugated) bile acids also included HDCA and HCA levels. The total concentration of primary, secondary, and 6α-hydroxylated species was determined by adding the values for all unconjugated and conjugated species (CDCA + CA, LCA + DCA, and HDCA + HCA, respectively). Because bile acid concentrations did not follow a normal distribution as shown by the Shapiro–Wilk test, the Wilcoxon matched-pairs signed-
ranks test was used for statistical analyses of the response to treatment in men and women separately, and also in both genders considered together. For comparison between men and women with respect to baseline bile acid profiles and response to treatment, the Wilcoxon/Mann–Whitney rank-sum test was used. Statistical analyses were performed using the JMP Statistical Discovery program, version 7.0.2 (SAS Institute, Cary, NC). Finally, analyses of covariance were used to compare the mean values of each log10-transformed bile acid variable between two or more independent groups, adjusted for body weight and body mass index (SAS version 9.2; SAS Institute).

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**CONFLICT OF INTEREST**

The authors declared no conflict of interest.
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