

Improved analysis of bile acids in tissues and intestinal contents of rats using LC/ESI-MS[§]

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Abstract To evaluate bile acid (BA) metabolism in detail, we established a method for analyzing BA composition in various tissues and intestinal contents using ultra performance liquid chromatography/electrospray ionization mass spectrometry (UPLC/ESI-MS). Twenty-two individual BAs were determined simultaneously from extracts. We applied this method to define the differences in BA metabolism between two rat strains, WKAH and DA. The amount of total bile acids (TBAs) in the liver was significantly higher in WKAH than in DA rats. In contrast, TBA concentration in jejunal content, cecal content, colorectal content, and feces was higher in DA rats than in WKAH rats. Nearly all BAs in the liver were in the taurine- or glycine-conjugated form in DA rats, and the proportion of conjugated liver BAs was up to 75% in WKAH rats. Similar trends were observed for the conjugation rates in bile. The most abundant secondary BA in cecal content, colorectal content, and feces was hyodeoxycholic acid in WKAH rats and ω -muricholic acid in DA rats. **■** Analyzing detailed BA profiles, including conjugation status, in a single run is possible using UPLC/ESI-MS. This method will be useful for investigating the roles of BA metabolism under physiological and pathological conditions.—Hagio, M., M. Matsumoto, M. Fukushima, H. Hara, and S. Ishizuka. Improved analysis of bile acids in tissues and intestinal contents of rats using LC/ESI-MS. *J. Lipid Res.* 2009. 50: 173–180.

Supplementary key words HPLC • ultra performance liquid chromatography • electrospray ionization mass spectrometry

Bile acids (BAs) are synthesized from cholesterol by various hepatic enzymes in the liver (1). They are indispensable compounds that absorb hydrophobic nutrients, including vitamins A and D, due to their amphipathic structure. Composition of primary bile acids (PBAs) depends on the species and changes over a lifetime (2–5). BAs are absorbed mainly from epithelial cells in the ileum and move back to the liver through the portal vein. The circulating BAs directly influence the neosynthesis of PBAs

in the liver via nuclear receptors (6). Some of the BAs flow into the large intestinal lumen and are converted to secondary bile acids (SBAs). In the large intestine, BAs affect cell kinetics of the epithelial cells. Hydrophobic BAs, such as deoxycholic acid (DCA) or lithocholic acid (LCA), have been reported to induce cell death in some cultured intestinal epithelial cell lines (7–9). Chenodeoxycholic acid (CDCA) enhances proliferation of certain cell lines (10), but the direct effect of each BA on epithelial cells depends on the cell line used. Moreover, in vivo, a variety of BAs exist at the same time in various tissues and intestinal contents, making it difficult to determine the precise influence of BAs on proliferation or survival of epithelial cells. In addition, various food components, such as dietary fibers and lipids, modulate BA levels or composition in intestinal contents and feces (11–15). The dietary interventions of BAs are also involved in development of colorectal cancer in various animal experiments (16–18). For clarifying the roles of dietary intervention to prevent diseases via modulating BAs, understanding the precise BA composition in the environment is critical.

Composition of BAs has been analyzed using gas chromatography (GC) or GC/mass spectrometry (GC/MS) techniques. Sample preparation for GC analysis is typically time-consuming owing to multiple steps, including methylation or trimethylsilylation, which lead to a loss in BAs at each step. Moreover, aliquots of the samples must be extracted separately to detect conjugated BAs in GC analysis. Compared with GC, HPLC is more prevalent for compositional analysis of BA (19, 20). Ando et al. (21) analyzed

Abbreviations: BA, bile acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LC/ESI-MS, liquid chromatography/electrospray ionization mass spectrometry; HDCA, hyodeoxycholic acid; LCA, lithocholic acid; MCA, muricholic acid; NDCA, nordeoxycholic acid; PBA, primary bile acid; SBA, secondary bile acid; SIR, selected ion recording; TBA, total bile acid; UDCA, ursodeoxycholic acid; UPLC, ultra performance liquid chromatography.

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serum BA composition in rats using HPLC/MS. Composition analysis of BAs can be improved further by using ultra performance liquid chromatography (UPLC) techniques, owing to better performance in terms of run-time and separation ability.

In this study, the goal was to establish a general protocol for BA extraction from various tissues and intestinal contents and to develop an analytical method for the determination of BA composition using UPLC/electrospray ionization mass spectrometry (ESI-MS). These methods could be used to clarify BA metabolism.

EXPERIMENTAL PROCEDURES

Chemicals

Cholic acid (5 β -cholanolic acid-3 α ,7 α ,12 α -triol, CA), α -muri-
 cholic acid (5 β -cholanolic acid-3 α ,6 β ,7 α -triol, α MCA), β -muri-
 cholic acid (5 β -cholanolic acid-3 α ,6 β ,7 β -triol, β MCA), ω -muri-
 cholic acid (5 β -cholanolic acid-3 α ,6 α ,7 β -triol, ω MCA), chenodeoxycholic acid
 (5 β -cholanolic acid-3 α ,7 α -diol, CDCA), deoxycholic acid (5 β -cholanolic
 acid-3 α ,12 α -diol, DCA), hyodeoxycholic acid (5 β -cholanolic acid-
 3 α ,6 α -diol, HDCA), ursodeoxycholic acid (5 β -cholanolic acid-3 α ,
 7 β -diol, UDCA), lithocholic acid (5 β -cholanolic acid-3 α -ol, LCA),
 taurocholic acid [5 β -cholanolic acid-3 α ,7 α ,12 α -triol-*N*-(2-sulpho-
 ethyl)-amide], tauro- α -muri-
 cholic acid [5 β -cholanolic acid-3 α ,
 6 β ,7 α -triol-*N*-(2-sulphoethyl)-amide], tauro- ω -muri-
 cholic acid [5 β -cholanolic acid-3 α ,6 α ,7 β -triol-*N*-(2-sulphoethyl)-amide], tauro-
 chenodeoxycholic acid [5 β -cholanolic acid-3 α ,7 α -diol-*N*-(2-sulpho-
 ethyl)-amide], taurodeoxycholic acid [5 β -cholanolic acid-3 α ,
 12 α -diol-*N*-(2-sulphoethyl)-amide], taurohyodeoxycholic acid [5 β -
 cholanolic acid-3 α ,6 α -diol-*N*-(2-sulphoethyl)-amide], tauro-
 lithocholic acid [5 β -cholanolic acid-3 α -ol-*N*-(2-sulphoethyl)-amide], glycocholic
 acid [5 β -cholanolic acid-3 α ,7 α ,12 α -triol-*N*-(carboxymethyl)-amide],
 glycochenodeoxycholic acid [5 β -cholanolic acid-3 α ,7 α -diol-*N*-
 (carboxymethyl)-amide], glycodeoxycholic acid [5 β -cholanolic
 acid-3 α ,12 α -diol-*N*-(carboxymethyl)-amide], glycohyodeoxycholic
 acid [5 β -cholanolic acid-3 α ,6 α -diol-*N*-(carboxymethyl)-amide],
 glyoursodeoxycholic acid [5 β -cholanolic acid-3 α ,7 β -diol-*N*-
 (carboxymethyl)-amide], glycolithocholic acid [5 β -cholanolic acid-
 3 α -ol-*N*-(carboxymethyl)-amide], and 23-nordeoxycholic acid
 (23-nor-5 β -cholanolic acid-3 α ,12 α -diol, NDCA) were purchased
 from Steraloids, Inc. (Newport, RI). The water used throughout
 the experiments was ion-exchanged and redistilled. HPLC grade
 acetonitrile, ethanol, and methanol were used for analyses.

Animals

The study was approved by the Hokkaido University Animal
 Committee, and the animals were maintained in accordance with
 Hokkaido University guidelines for the care and use of laboratory
 animals. Male WKAH/Hkm Slc and DA Slc rats (6 weeks old, $n =$
 8) were purchased from Japan SLC, Inc. (Hamamatsu, Japan).
 The rats were kept in an air-conditioned room at $22 \pm 2^\circ\text{C}$ and
 $55 \pm 5\%$ humidity. The lighting period was from 08:00 to 20:00.
 The rats had free access to a purified diet and water for 7 weeks.
 The diet composition was as follows (22): 52.95% dextrin, 20%
 casein, 10% sucrose, 7% soybean oil, 5% cellulose, 3.5% mineral
 mixture (AIN 93G), 1% vitamin mixture (AIN 93G), 0.3% L-cystine,
 and 0.25% choline chloride. The rats were anesthetized using a
 ketamine and xylazine mixture (60 $\mu\text{l}/100$ g body weight) con-
 taining 50 mg/ml ketamine and 8.64 mg/ml xylazine. A cannula-
 tion was placed into the bile duct of the anesthetized rats, and the
 bile was collected for 15 min via the cannula. Arterial blood was

collected from the aorta abdominalis. Other samples collected
 were from the liver, intestinal contents (jejunum, ileum, cecum,
 and colorectum), and feces. Feces were collected for a day at the
 end of the experimental period. Arterial blood plasma was
 obtained immediately after the collection by centrifugation at
 1,000 g for 10 min at 4°C . All samples were stored at -80°C
 until analysis.

Sample preparation for UPLC/ESI-MS

Stored liver, intestinal contents, and feces were freeze-dried
 and ground thoroughly. One milliliter of ethanol was added to
 100 mg of the ground samples to extract BAs. Nordeoxycholic
 acid (NDCA) (25 nmol) was added as an internal standard to
 each sample. The samples were subjected to sonication (constant,
 40 cycles, control 2.5, twice for 10 s; Ultra S Homogenizer VP-15S;
 Taitec Corp., Saitama, Japan) and then heated at 60°C for 30 min
 in a water bath. After cooling to room temperature, the samples
 were heated at 100°C in a water bath for 3 min and centrifuged at
 1,600 g for 10 min at 15°C . The supernatants were then collected.
 To the precipitates, 1 ml of ethanol was added and mixed vig-

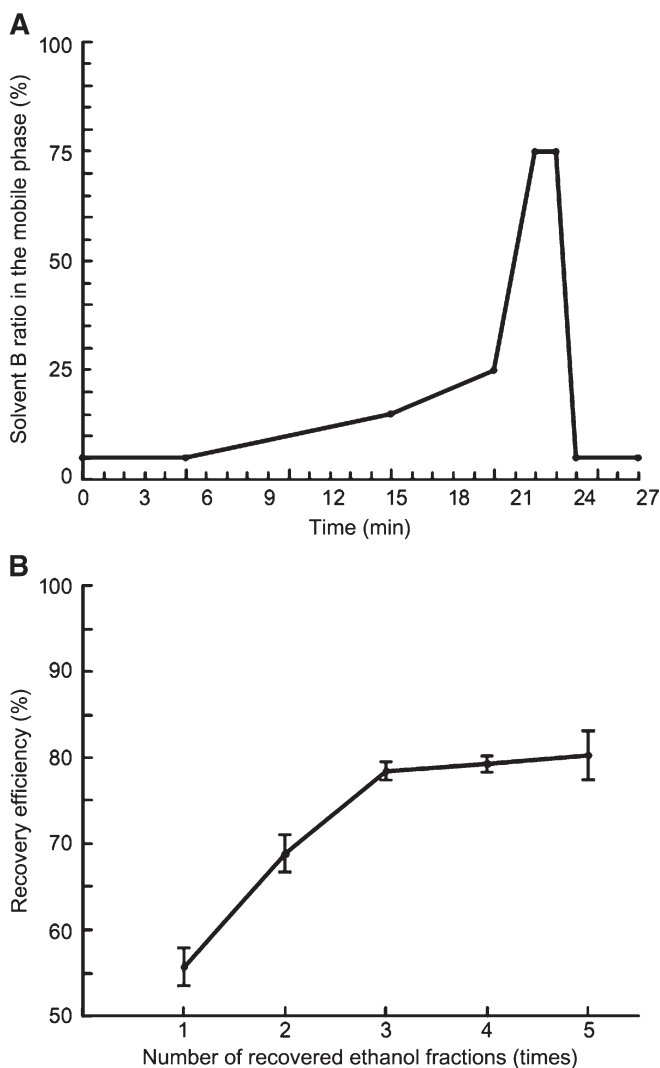


Fig. 1. A: Elution profile (change in Solvent B ratio) for ultra performance liquid chromatography (UPLC) analysis of bile acids (BAs). B: Nordeoxycholic acid (NDCA) recovered by repeated extraction from rat fecal suspensions (added NDCA, 25 nmol/100 mg feces). Values are expressed as the mean \pm SD.

rously by vortex for 1 min. The samples were centrifuged at 11,200 *g* for 1 min, and the supernatants were collected. This extraction was repeated once more. The pooled extracts from a sample were evaporated, and then 1 ml of methanol was added to the dried extracts. The methanol extracts were purified using an HLB cartridge (Waters, Milford, MA) according to the manufacturer's instructions. The 100 μ l of plasma or 50 μ l of bile samples was freeze-dried and extracted as described above without heating.

Analysis using UPLC/ESI-MS

Liquid chromatography (LC) separation was performed using an Acquity UPLC system (Waters) with a gradient elution from a BEH C18 column (1.7 μ m, 100 mm \times 2.0 mm ID; Waters) and maintained at 40°C and a flow rate of 400 μ l/min. The auto sampler was kept at 15°C. The sample injection volume was 5 μ l. Solvent A was acetonitrile-water (20:80) containing 10 mM ammonium acetate. Solvent B was acetonitrile-water (80:20) containing 10 mM ammonium acetate. The gradient program is shown in Fig. 1A. The column eluent was introduced into the MS.

MS analysis was performed using a Quattro Premier XE quadrupole tandem MS (Waters) equipped with an ESI probe in negative-ion mode. A capillary voltage of -3,200 V, a source temperature of 120°C, and a desolvation temperature of 400°C were used. The cone voltage was 35 V for unconjugated, glycine-conjugated, and taurine-conjugated BAs. The desolvation and cone gas flow were 800 l/h and 50 l/h, respectively. Selected ion recording (SIR) was performed by examining product ions of the deprotonated molecules from each BA. For unconjugated BAs, the *m/z* values of the product ions from mono-, di-, and tri-hydroxylated BAs were 375.6, 391.6, and 407.6, respectively. For glycine-conjugated BAs, *m/z* values of the product ions from mono-, di-, and tri-hydroxylated BAs were selected as 432.6, 448.6, and 464.6, respectively. For taurine-conjugated BAs, the *m/z* values of the product ions from mono-, di-, and tri-hydroxylated BAs were selected as 482.7, 498.7, and 514.7, respectively. For the internal standard, the *m/z* of NDCA was 377.5. Concentrations

of individual BAs were calculated from the peak area in the chromatogram detected with SIR relative to the internal standard, NDCA.

Statistics

Differences between treatment groups were determined using Scheffe's multiple range test. A probability of less than 0.05 was considered significant.

RESULTS

Extraction of BA

In a preliminary experiment, we identified the optimal temperature and time of extraction for DCA from rat feces. The heating steps in the extraction of BA are usually necessary to reduce hydrophobic interactions within the samples. As a result, the most effective extraction efficiency was established, in which the BAs were extracted at 60°C for 30 min and 100°C for 3 min in the first and second step, respectively. Next, we evaluated repeated extraction with ethanol for recovery of NDCA added to the fecal suspension. To qualify the recovery efficiency, the optimum number of extraction steps was investigated (Fig. 1B). In the repeated extraction, we simply added ethanol to the precipitates without heating and pooled the whole recovered ethanol fractions. Three rounds of ethanol extraction were found to be sufficient for maximum recovery of the added NDCA.

Analysis of BA using UPLC/ESI-MS

We selected 22 different BAs containing seven types of taurine conjugates and six types of glycine conjugates, in

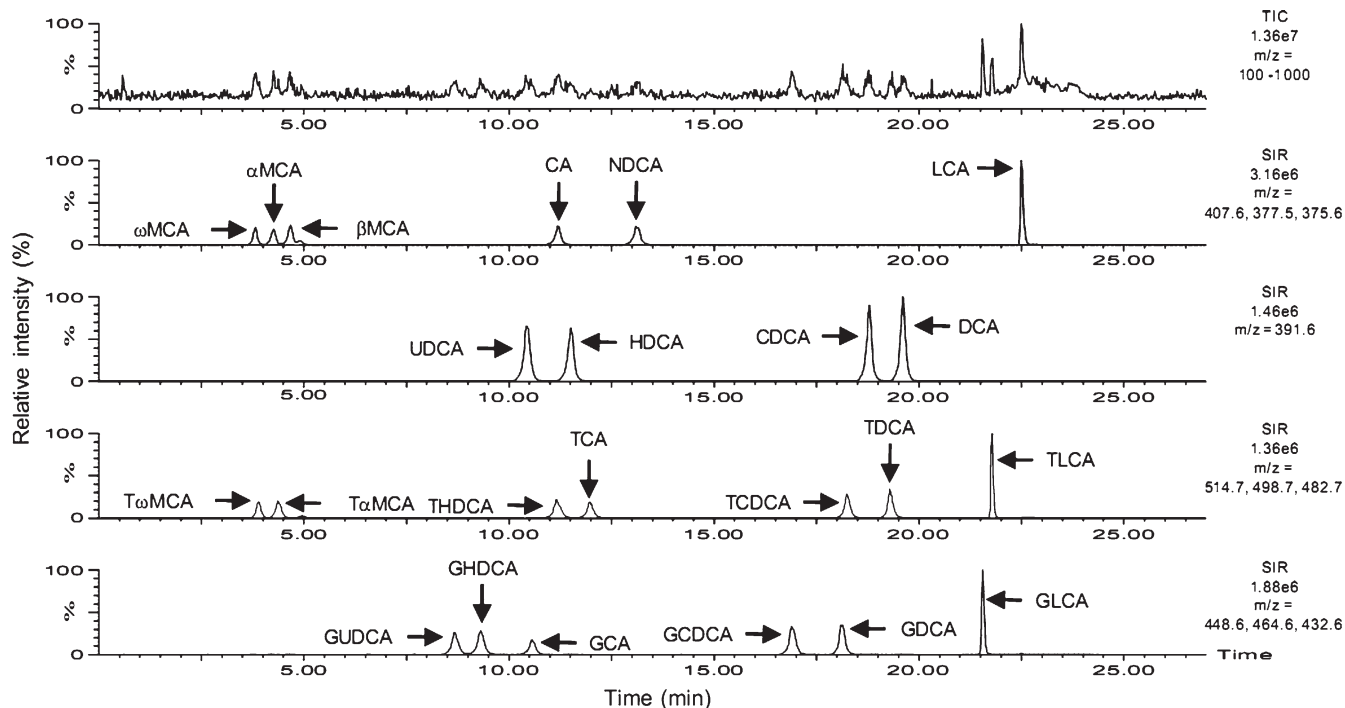


Fig. 2. Selected ion recording (SIR) chromatograms for BA standard solutions (50 μ M each) from UPLC analysis.

addition to the internal standard, NDCA. All BA standards were separated well in the same LC gradient (Fig. 1A) with the ESI-MS program (Fig. 2). The BAs in various biological samples were analyzed successfully within 30 min using UPLC/ESI-MS. Representative chromatograms of BAs in the ethanol extracts of bile and feces of DA rats are shown in Fig. 3. In bile, nearly all of the BAs were conjugated with taurine or glycine (Fig. 3A). In feces, a large amount of hydroxycholeic acid (HDCA) and ω -muricholic acid

(ω MCA) was detected. Other BAs such as β MCA, CA, LCA, UDCA, and DCA were also found (Fig. 3B).

Accuracy of BA analysis

To confirm the precision of BA quantification using MS, the ranges of detected area of 23 BAs in MS chart were investigated by five successive analyses of single standard mixture sample. As a result, the ratios of standard deviation (SD) against average values of all BA concentrations

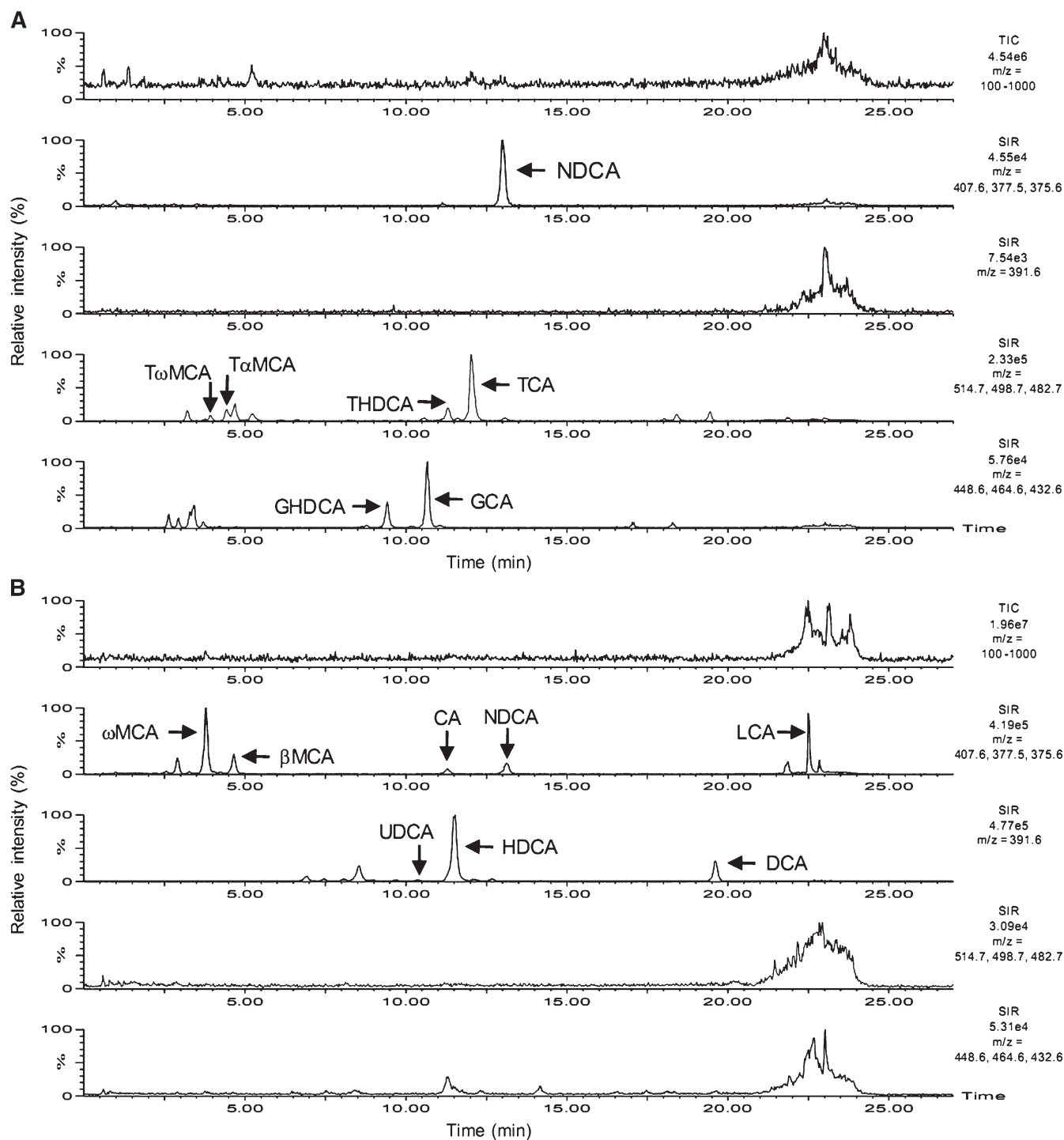


Fig. 3. Representative chromatograms of BAs in (A) bile and (B) feces of DA rats detected in SIR mode.

analyzed ranged from 1.79% (DCA) to 7.12% (HDCA, glycolithocholic acid). As a biological sample, unconjugated BAs in feces of DA rats were analyzed. The ratios of SD against the average values of nine unconjugated BAs ranged from 1.04% (HDCA) to 9.58% (CDCA). To confirm the differences of extraction efficiency among BAs, recovery tests were conducted, adding the exogenous BAs to the feces of DA rats at the first step of extraction. Selected BAs were ω MCA, HDCA, and LCA, represented as tri-, di-, and mono-hydroxylated BA, respectively. Each BA was supplemented with twice the amount of the original concentrations in the feces. At the same time, NDCA was also added as the internal standard. The recovery ratios of these exogenous standards calculated from each raw area in the chart were 90.7, 90.8, and 89.4% in ω MCA, HDCA, and LCA, respectively (n = 6). In cases in which these ratios were compensated by the values of NDCA, the recovery ratios were 97.8%, 99.1%, and 96.9%, respectively. Taken together, reliability of the BA analysis was confirmed when calculated in combination with the internal standard.

BA analysis in the liver and bile

Using this method, we measured 22 BAs in the same extracts from tissues and intestinal contents of WKAH and DA rats. Composition of BAs in the liver and bile is shown in **Table 1**. Total bile acids (TBAs) are expressed as the

TABLE 1. Bile acid concentrations in the liver and bile of WKAH and DA rats

	Liver		Bile	
	WKAH	DA	WKAH	DA
	<i>nmol/g dry tissue</i>		<i>μmol/ml</i>	
Unconjugated				
CA	18.5 ± 6.6	ND ^a	1.00 ± 0.27	0.04 ± 0.02 ^a
αMCA	9.2 ± 3.2	ND ^a	0.12 ± 0.04	<0.01 ^a
βMCA	24.5 ± 7.4	ND ^a	0.09 ± 0.03	<0.01 ^a
ωMCA	6.6 ± 2.1	ND ^a	0.07 ± 0.02	ND ^a
HDCA	16.3 ± 4.9	ND ^a	0.01 ± 0.01	ND ^a
UDCA	0.9 ± 0.3	ND ^a	ND	ND
CDCA	ND	ND	ND	ND
DCA	ND	ND	ND	ND
LCA	1.4 ± 1.1	1.1 ± 0.1	ND	ND
Taurine-conjugated				
CA	89.4 ± 22.3	61.0 ± 9.9	5.05 ± 0.91	6.68 ± 0.74
αMCA	64.0 ± 21.0	46.4 ± 5.8	3.92 ± 2.19	6.07 ± 1.83
ωMCA	8.8 ± 2.3	6.4 ± 1.0	0.76 ± 0.30	0.73 ± 0.11
HDCA	23.8 ± 3.6	ND ^a	0.81 ± 0.15	0.51 ± 0.15
CDCA	ND	2.6 ± 0.9 ^a	0.11 ± 0.02	0.33 ± 0.03 ^a
DCA	ND	5.2 ± 1.4 ^a	0.22 ± 0.03	0.31 ± 0.04
LCA	ND	ND	ND	ND
Glycine-conjugated				
CA	ND	ND	0.05 ± 0.01	0.38 ± 0.15 ^a
HDCA	ND	ND	0.01 ± 0.01	0.04 ± 0.02
UDCA	ND	ND	ND	<0.01 ^a
CDCA	ND	ND	ND	0.01 ± 0.01 ^a
DCA	ND	ND	ND	ND
LCA	ND	ND	ND	ND
TBA	263.4 ± 41.1	122.8 ± 12.3 ^a	12.23 ± 3.27	15.13 ± 2.49

CA, cholic acid; MCA, muricholic acid; HDCA, hyodeoxycholic acid; UDCA, ursodeoxycholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; TBA, total bile acid; ND, not detected. Values are expressed as means with SEM.

^aSignificant difference from the data in WKAH (*P* < 0.05, n = 8).

sum of the values of each BA analyzed. The concentration of TBA in the liver of WKAH rats was significantly higher than that of DA rats. No significant difference was observed in TBA concentration in bile between the rat strains. Large amounts of unconjugated BAs were detected in both the liver and bile of WKAH rats.

BA metabolism from the liver to feces

We also evaluated BA metabolism in various tissues and intestinal contents among the liver, bile, intestinal contents (jejunum, ileum, cecum, and colorectum), and feces. **Figure 4A** shows the changes in conjugation ratio, indicating the proportion of taurine- and glycine-conjugated BA concentrations as a function of TBA. The conjugation ratio of BAs in DA rats was maintained at nearly 100% in both the liver and bile (Fig. 4A), but the conjugation level for WKAH rats was about 74% and 85%, respectively. In both strains, the conjugation ratio decreased gradually from bile to cecal content; the level was about 1% in the cecal content. The conjugation ratio was nearly constant from cecal content to feces. In WKAH rats, the proportion of conjugated BAs from liver to ileum was significantly lower than that in DA rats.

Figure 4B shows changes in the SBA ratio, which indicates the proportion of SBA (BAs except for CA, CDCA, αMCA, and βMCA in spite of the conjugation status) concentrations relative to TBAs. The SBA ratio from liver to ileal content in both strains was maintained at a low level (Fig. 4B), but the ratio increased dramatically to about 85% in the cecal content. Significant differences in the SBA ratio were observed between the strains, particularly in the liver, bile, and jejunal and colorectal contents. Overall, the SBA ratio in WKAH rats tended to be higher than that in DA rats.

TBA concentrations in the intestinal contents and feces, shown in Fig. 4C, were significantly higher in DA rats than in WKAH rats in the intestinal contents, except for the ileum. A considerable reduction of TBAs was observed in the large intestine in both strains.

BA composition in the large intestine and arterial blood plasma

To investigate the influence of BAs on the intestinal epithelium in the large intestine, one must understand the BA concentration in the surrounding environment, in both the intestinal contents and the plasma. In PBAs, a certain amount of βMCA was found in the cecal content in both DA and WKAH rats (see supplementary Fig. 1A). Among SBAs, UDCA and LCA concentrations were lower than others (ωMCA, HDCA, and DCA). In particular, the ωMCA concentration in the cecal contents, colorectal contents, and feces was prominently higher in DA rats than in WKAH rats. The most abundant BA was HDCA in the large intestine and feces of WKAH rats. DCA concentration decreased gradually through the large intestine. There was no difference of BA composition among large intestinal contents in each strain. Various BAs in plasma were also analyzed (see supplementary Fig. 1B). Glycine-conjugated BAs in plasma were not detected. Unconjugated BA

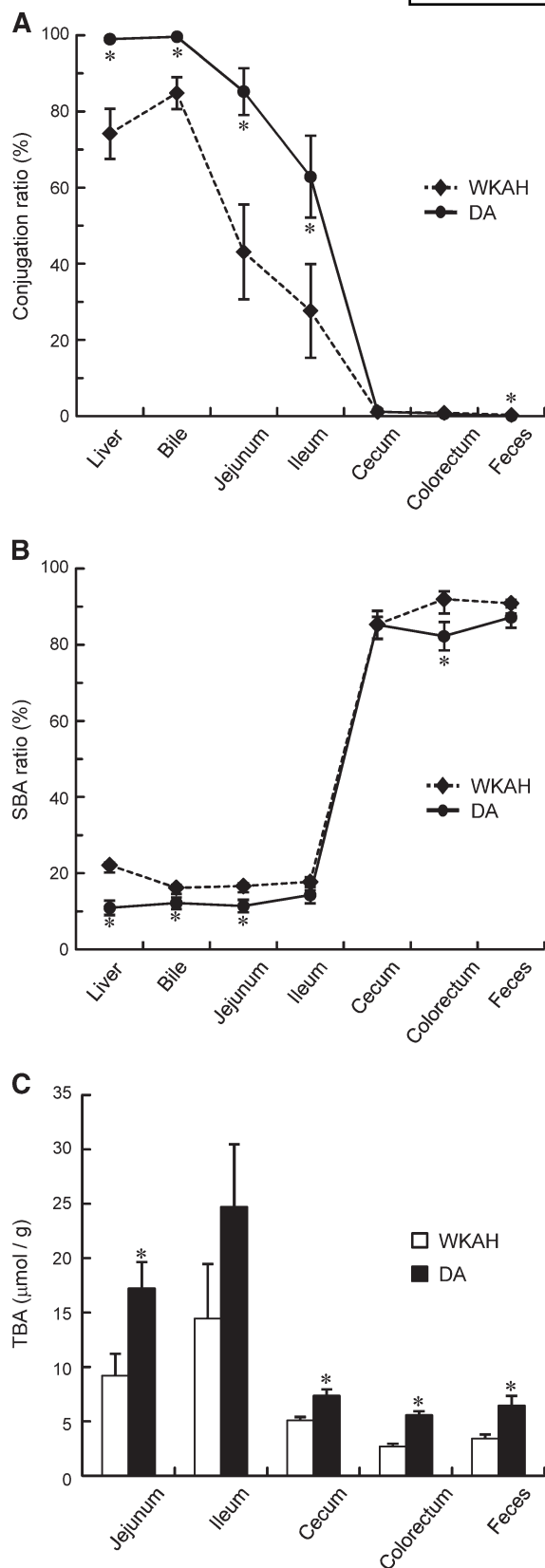


Fig. 4. A: Changes in the conjugation ratio of BAs from liver to feces in WKAH and DA rats. B: Changes in the secondary bile acid ratio from liver to feces in WKAH and DA rats. C: Amount of total bile acids in intestinal contents and feces. Values are expressed as mean with SEM. An asterisk indicates a significant difference from the values in WKAH ($P < 0.05$, $n = 8$).

amounts in plasma were significantly higher in WKAH rats than in DA rats. The amount of HDCA in WKAH rats was about 20-fold that in DA rats. Unconjugated BA concentrations seemed to be higher than taurine-conjugated concentrations in WKAH rats.

Growth, tissue weights, bile flow, and liver lipids in WKAH and DA rats

Clearly, the growth characteristics and concentration of liver lipids were different between WKAH and DA rats (Table 2). Body weight gain in WKAH rats was significantly higher than in DA rats, along with the food intake. Relative weights of the mesenteric, perirenal and dorsal, and epididymal adipose tissues in WKAH rats were significantly greater than those of DA rats. In contrast, the relative weight of the intestine (jejunum, ileum, cecum, and colorectum) was greater in DA rats than in WKAH rats. The triglyceride concentration in the liver was much higher in WKAH rats compared with DA rats, although the total cholesterol in the liver was lower in WKAH rats.

DISCUSSION

The analysis of BA composition in biological fluids using LC/MS techniques has been reported previously, for example, in serum and urine (23). To understand the precise metabolism of BAs, information on BA composition in other organs and intestinal contents is clearly essential. To the best of our knowledge, no previous report had shown a BA analysis of samples containing various solid materials using LC/MS techniques. Thus, one of the aims of this study was to develop a general method for extracting BAs from various biological samples containing solid constituents. We first lyophilized and ground each of the biological samples, except plasma and biles, to extract BAs

TABLE 2. Growth, tissue weights, bile flow, and liver lipids in WKAH and DA rats

	WKAH	DA
Rat growth (g)		
Initial body weight	120.4 ± 2.7	112.2 ± 2.2 ^a
Body weight gain	249.1 ± 15.6	97.7 ± 4.0 ^a
Food intake	890.3 ± 12.9	580.3 ± 9.5 ^a
Tissue weight (g/100 g body weight)		
Liver	3.604 ± 0.063	3.025 ± 0.060 ^a
Jejunum	0.325 ± 0.013	0.436 ± 0.013 ^a
Ileum	0.254 ± 0.007	0.280 ± 0.006 ^a
Cecum	0.200 ± 0.007	0.440 ± 0.037 ^a
Colorectum	0.242 ± 0.005	0.332 ± 0.013 ^a
Mesenteric adipose tissue	1.652 ± 0.038	0.894 ± 0.045 ^a
Perirenal and dorsal adipose tissue	2.687 ± 0.038	1.102 ± 0.074 ^a
Epididymal adipose tissue	2.117 ± 0.039	1.180 ± 0.029 ^a
Bile flow (ml/100 g body weight/h)	0.348 ± 0.041	0.327 ± 0.010
Liver lipids (μmol/g dry tissue)		
Total cholesterol	15.32 ± 0.33	16.82 ± 0.35 ^a
Triglyceride	123.05 ± 6.07	47.27 ± 2.90 ^a

Values are expressed as means with SEM.

^aSignificant difference from the data in WKAH ($P < 0.05$, $n = 8$).

effectively from samples containing solid materials. Although chloroform with methanol is generally used in lipid extractions, it is not required in BA extraction because BAs are amphipathic. Ethanol extraction is another option. Furthermore, we heated extraction samples (except plasma and bile) in successive extraction steps to reduce hydrophobic interactions among extraction materials. In a preliminary experiment, we confirmed that no BA was destroyed during the heating and sonication steps. Taken together, BA extraction from various biological samples has been established. In the previous reports, about 1 h was required for BA analysis using LC/MS (21). We can reduce the runtime by nearly one-half to analyze a broad spectrum of BAs, owing to the superior performance provided by UPLC.

BAs contribute to lipid absorption in the small intestine and are absorbed via epithelial cells in the distal ileum. Residual BAs in the intestinal lumen move into the large intestine and are excreted with feces. Some of these PBAs are converted into the cognate SBAs by bacteria in the large intestine. PBAs in humans are CA and CDCA. In contrast, MCAs are produced in rats from CDCA, owing to the existence of 6 β -hydroxylase in the liver (24). Because the 7 position of 5 β -cholic acid can be hydroxylated in both the α and β directions, rats produce four types of PBAs in their liver (25, 26). Thus, the BA composition in rats is more complicated than in humans, especially in the large intestine.

In general, deconjugation of BAs occurs in the large intestine (27, 28). The conjugation rates in the intestinal contents decreased gradually from the bile to cecum. Almost all BAs were deconjugated in the cecal content (Fig. 4A). Certain intestinal bacteria may be contributing to this deconjugation, even in the jejunal and ileal contents. The SBA ratio, however, was kept at a low level from the liver to the ileum, and increased dramatically in the cecal content (Fig. 4B). This observation is in agreement with previous reports (29). These results indicate that deconjugation of BAs is followed by conversion of the PBAs to SBAs. TBA concentration decreased greatly in the large intestinal contents, as shown in Fig. 4C. The decrease in TBAs in the large intestine was thought to be due to enterohepatic circulation.

BAs are involved in many diseases of the large intestine, depending on their chemical structure and conjugation status (30). The number of aberrant crypt foci induced by γ -ray or 1,2-dimethylhydrazine was greater in WKAH rats than in DA rats (31), suggesting different BA profiles in the environment. In this study, we verified the BA profiles in various tissues, intestinal contents, and feces between the two strains of rat without any pathological treatment. The conjugation rates of BAs were very low in the small intestinal contents of WKAH rats compared with DA rats (Fig. 4A). Expression of BA-CoA:amino acid *N*-acetyltransferase, the sole enzyme responsible for conjugation of BAs with taurine or glycine (1, 32), may be different between the two strains. DCA induces apoptosis in H508 cells derived from human colon cancer, but both the taurine and glycine conjugates did not induce apoptosis (33). This suggests a protective effect of SBAs on the epithelial cells by taurine or glycine conjugation, indicating the tumor resistance of DA rats.

In conclusion, we established an improved method for analyzing BA profiles in various biological samples using UPLC/ESI-MS, which facilitates comparison of BA composition in tissues or intestinal contents. In addition, this technique could be applied to clarify pathological changes in BA profiles in disease, leading to a better understanding of BA metabolism.

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