

Bile acid patterns in meconium are influenced by cholestasis of pregnancy and not altered by ursodeoxycholic acid treatment

C M P Rodrigues, J J G Marín, D Brites

Abstract

Background—Data on meconium bile acid composition in newborn babies of patients with intrahepatic cholestasis of pregnancy (ICP) are relatively scant, and changes that occur on ursodeoxycholic acid (UDCA) administration have not been evaluated.

Aims—To investigate bile acid profiles in meconium of neonates from untreated and UDCA treated patients with ICP. Maternal serum bile acid composition was also analysed both at diagnosis and delivery to determine whether this influences the concentration and proportion of bile acids in the meconium.

Patients/Methods—The population included eight healthy pregnant women and 16 patients with ICP, nine of which received UDCA (12.5–15.0 mg/kg body weight/day) for 15±4 days until parturition. Bile acids were assessed in the meconium by gas chromatography-mass spectrometry and in maternal serum by high performance liquid chromatography. **Results**—Total bile acid and cholic acid concentrations in the meconium were increased ($p < 0.01$) in newborns from patients with ICP (13.5 (5.1) and 8.4 (4.1) $\mu\text{mol/g}$ respectively; mean (SEM)) as compared with controls (2.0 (0.5) and 0.8 (0.3) $\mu\text{mol/g}$ respectively), reflecting the total bile acid and cholic acid levels in the maternal serum ($r = 0.85$ and $r = 0.84$, $p < 0.01$). After UDCA administration, total bile acid concentrations decreased in the mother (~3-fold, $p < 0.05$) but not in the meconium. UDCA concentration in the meconium showed only a 2-fold increase after treatment, despite the much greater increase in the maternal serum ($p < 0.01$). Lithocholic acid concentration in the meconium was not increased by UDCA treatment.

Conclusions—UDCA administration does not influence the concentration and proportion of bile acids in the meconium, which in turn are altered by ICP. Moreover, this beneficial treatment for the mother does not increase meconium levels of potentially toxic metabolites of UDCA such as lithocholic acid.

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Keywords: bile acids; cholestasis; pregnancy; cholic acid; meconium; ursodeoxycholic acid therapy

Intrahepatic cholestasis of pregnancy (ICP) is generally considered benign to the mother although it often causes pruritus and biochemical cholestasis.¹⁻³ Increased fetal distress, premature deliveries, and perinatal mortality and morbidity have been described⁴⁻⁶ and raised some concerns. Moreover, high frequency of meconium staining of the amniotic fluid may be considered a sign of poor prognosis for the fetus.⁵⁻⁷ It may be possible that harmful effects on fetal wellbeing result from high bile acid levels in amniotic fluid and serum cord blood, reflecting an appreciable increase in bile acids in maternal serum,⁸⁻¹¹ as well as from a reduced ability of the fetus to eliminate bile acids across the placenta.¹²

Ursodeoxycholic acid (UDCA) administration during ICP has been shown to improve both pruritus and liver function tests, correct maternal serum bile acid profile,¹³⁻²¹ and decrease passage of bile acids to the fetus.²²⁻²³ Furthermore, a valuable contribution to favourable fetal outcome is the ability of UDCA to reverse the impairment of bile acid transport across the trophoblast.¹²

An investigation of the bile acid pattern in meconium of newborn babies of patients with ICP may give some insight into whether cholestasis in the mother is a governing factor in meconium bile acid composition. Meconium comprises a concentrate of metabolites of both fetal and maternal origin, in which the bile acid composition is influenced by the presence of bile in the fetal duodenum and by the ingestion of amniotic fluid. Moreover, equilibrium between the fetal bile acid pool and the intestinal contents appears to be restricted because of the poor fetal enterohepatic circulation of bile acids. The occurrence of a pattern of unusual bile acids in the meconium, similar to that of patients with cholestatic liver disease, has been attributed to physiological cholestasis during the developmental phase.²⁴⁻²⁸ It is therefore tempting to speculate that UDCA may have some beneficial effects on the cholestatic pattern of bile acids in the meconium during ICP.

Methods

STUDY PROTOCOL

This study was conducted in collaboration with Santa Maria Hospital, Garcia de Orta Hospital, and Dr Alfredo da Costa Maternity Ward from 1994 to 1997. Eight healthy volunteers at

Abbreviations used in this paper: ICP, intrahepatic cholestasis of pregnancy; UDCA, ursodeoxycholic acid; GC-MS, gas chromatography-mass spectrometry.

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full term gestation were enrolled and considered as the control group. None had a history of hepatic disease or pre-eclampsia. Sixteen patients with ICP were included in the study. Inclusion criteria were as previously described^{4,20}: (a) severe pruritus; (b) total bile acids above 11 $\mu\text{mol/l}$, cholic acid percentage higher than 40%, and increased alanine aminotransferase or conjugated bilirubin; (c) absence of any other known disease that could cause liver malfunction. According to the severity of the ICP disease, seven patients were given classical palliative treatments (less severe, "non-UDCA group"), and nine received UDCA (more severe, "UDCA group") at 12.5–15.0 mg/kg body weight/day beginning 20 days or less (15 \pm 4 days, 9–20 days) before term. Pruritus assessment and its changes during UDCA treatment were based on complaints from the patients. Four women in the UDCA group had previously suffered from cholestasis of pregnancy. The drug was discontinued at delivery and fasting maternal serum as well as meconium samples were collected. Meconium and maternal serum samples were also collected from eight uncomplicated pregnancies at term. Specimens were stored under a nitrogen environment at -20°C , after flash-freezing in liquid nitrogen, until analysed.

Informed consent was obtained from each patient and the study protocol conformed with the ethical guidelines of the 1975 Declaration of Helsinki.

ANALYTICAL TECHNIQUES

Liver function tests

Routine laboratory methods were used to determine serum biochemical markers of cholestasis including aspartate aminotransferase, alanine aminotransferase, γ -glutamyltranspeptidase, alkaline phosphatase, and total and conjugated bilirubin.

Bile acid analysis in maternal serum

Bile acids were measured in maternal serum, collected at both diagnosis and delivery, by high performance liquid chromatography and an enzymic-fluorimetric assay.

Bile acid conjugates—Individual amidated bile acids were determined by high performance liquid chromatography, essentially as described by Labbé *et al.*²⁹ with minor modifications. Briefly, after liquid-solid extraction using Sep-Pak C_{18} cartridges (Waters Corp, Milford, Massachusetts, USA),³⁰ separation of the principal glycine and taurine conjugated species in the maternal serum was achieved using a Shimadzu liquid chromatograph system, equipped with a variable wavelength ultraviolet detector and housing a reverse-phase Li-Chrospher 100 RP-8 endcapped column (Merck, Darmstadt, Germany).

Unconjugated bile acids—These species were evaluated using an enzymic-fluorimetric method,^{31,32} after solid-phase extraction and thin layer chromatography using silica gel plates.^{33–35} Unconjugated UDCA in serum was not individually quantified because of the diffi-

culty of separating it from chenodeoxycholic acid during thin layer chromatography.

Bile acid analysis in meconium—Bile acids were assessed in meconium by gas chromatography-mass spectrometry (GC-MS) after liquid-solid extraction, hydrolysis, isolation by lipophilic anion exchange chromatography, and conversion to methyl ester-trimethylsilyl ether derivatives.

Total and individual bile acids—Meconium samples were weighed (0.1–0.5 mg), and 80% methanol (50 ml) was added. All samples were sonicated for 30 minutes, refluxed for two hours, and then cooled and filtered.³⁶ The residue was resuspended in 50 ml chloroform/methanol (1:1, v/v), refluxed for one hour, and filtered. The combined extracts were taken to dryness under N_2 and the dried extract was resuspended in 80% methanol. Exactly one fifth of the extract was taken, and the internal standard, nordeoxycholic acid (5 or 10 μg), was added. The sample was diluted with distilled water and passed through a Sep-Pak C_{18} cartridge. Bile acids were recovered by elution with methanol, and hydrolysis of conjugated species was performed as previously described.^{37,38} In short, solvolysis was carried out in a mixture of methanol, distilled tetrahydrofuran, and 1 M trifluoroacetic acid in dioxane, heated to 45°C for two hours. Enzymic hydrolysis was achieved by overnight incubation with choloylglycine hydrolase (Sigma Chemical Co, St Louis, Missouri, USA) at 37°C . After hydrolysis, bile acids were extracted by passage of the sample through a Sep-Pak column and recovered by elution with methanol. The unconjugated species, after hydrolysis, were isolated and separated from neutral sterols by anion exchange chromatography on diethylaminohydroxypropyl-Sephadex LH-20³⁹ (Lipidex-DEAP; Packard Instrument Co, Groningen, The Netherlands). Recovery was achieved by elution with 0.1 M acetic acid in 72% ethanol (7 ml) and this fraction was evaporated to dryness. Methyl ester-trimethylsilyl ether derivatives were prepared^{40,41} and purified⁴² before separation by GC. GC-MS analysis was carried out using an HP 5890 series II gas chromatograph connected to an HP 5972 mass spectrometer. Identification of bile acids was based on the GC retention index relative to a homologous series of *n*-alkalis and the mass spectrum compared with authentic standards. When these were not available, identification was tentatively based on the predicted fragmentation patterns for specific bile acid structures.⁴³ Quantification of bile acids was achieved using GC, by comparing the peak height response of the individual bile acids with that obtained from the internal standard.

Bile acid conjugates—Six of the meconium samples collected from newborns of patients with ICP treated or not with UDCA (three from each group, including a low, medium, and high total bile concentration sample) were analysed

Table 1 Clinical data on patients with intrahepatic cholestasis of pregnancy

	Non-UDCA	UDCA
Age (years)	32.4 (4.2) (26–36)	30.8 (4.8) (23–35)
Recurrence	0	4
Gemelar pregnancy	1	0
Onset of pruritus (weeks)	33.1 (3.2) (28–35)	28.7 (4.0) (26–34)*
Gestational age at diagnosis (weeks)	35.4 (1.8) (33–37)	32.0 (2.1) (29–35)**
Gestational age at delivery (weeks)	36.5 (2.2) (34–39)	36.7 (0.4) (35–39)
Green amniotic fluid	2	3
Newborns		
Birth weight (g)	2870 (660) (2110–3670)	2902 (232) (2490–3380)
Apgar score		
1 minute	8.6 (0.9)	9.0 (0.0)
5 minutes	9.8 (0.5)	10.0 (0.0)

Patients with intrahepatic cholestasis of pregnancy received classical palliative therapy (non-UDCA group; n = 7) or were treated with ursodeoxycholic acid (UDCA) until labour (UDCA group; n = 9). Values are expressed as mean (SD) (respective range).

*p<0.05 and **p<0.01, v non-UDCA.

to evaluate the extent of bile acid conjugation. In addition, unconjugated and conjugated species were studied in three meconium samples from healthy newborns delivered from uncomplicated pregnancies. Methanolic extracts diluted to 72% methanol were passed through a column of Lipidex-DEAP, and unconjugated bile acids and their conjugates (glycine, taurine, and sulphate) were recovered by stepwise elution of the gel bed with appropriate buffers.⁴⁴ After evaporation, conjugated species were hydrolysed, recovered, and converted to methyl ester-trimethylsilyl ether derivatives as described above.

STATISTICAL ANALYSIS

Data were compared using the Wilcoxon non-parametric rank sum test or the unpaired two-tailed Student's *t* test performed on the basis of equal or unequal variance as appropriate. Results are expressed as mean (SEM) or mean (SD), as indicated. The relation between maternal serum and meconium bile acid composition was examined by linear regression analysis. p<0.05 was considered statistically significant.

Results

CLINICAL OBSERVATIONS

Statistical differences were noticed with respect to the onset of pruritus (33.1 (3.2) v 28.7 (4.0) weeks of gestation, p<0.05) as well as to the gestational age at diagnosis (35.4 (1.8) v 32.0 (2.1) weeks, p<0.01) between patients with ICP receiving classic palliative treatment (non-

UDCA group) and those treated with UDCA (UDCA group) (table 1). The poor prognosis associated with an earlier onset of the disease led to the decision to start UDCA treatment in these patients and therefore justifies the statistical differences between the groups. No major side effects were reported in the nine patients studied during UDCA treatment. Compliance was assessed from the increased percentage of UDCA in the serum (higher than 18%), and no drop outs were recorded during the treatment period. Itching disappeared or was appreciably reduced during UDCA treatment. All newborns were healthy and no differences were noticed for birth weight and Apgar scores when neonates from women included in the UDCA and non-UDCA groups were compared, suggesting a potential benefit of UDCA administration. Pruritus vanished and laboratory tests became normal within one and two weeks of labour respectively. Table 1 gives other clinical observations.

Patients with ICP showed significant increases (p<0.01) in serum total bile acid concentrations (33.3 (11.4) v 5.0 (0.3) $\mu\text{mol/l}$ in control) and cholic acid percentage (61.3 (4.9) v 35.2 (1.1)%), as well as in alanine aminotransferase activity (324 (155) v 24 (4) IU/l) and conjugated bilirubin levels (0.42 (0.12) v 0.13 (0.01) mg/dl). In addition, γ -glutamyltranspeptidase activity was elevated in patients with ICP (34 (10) v 9 (1) IU/l, p<0.05). The group treated with UDCA showed significantly (p<0.05) lower serum total bile acid concentrations (13.0 (2.2) $\mu\text{mol/l}$), cholic acid percentage (23.3 (3.4)%), alanine aminotransferase (49 (23) IU/l), and γ -glutamyltranspeptidase activity (8 (1) IU/l), but not conjugated bilirubin concentration (0.39 (0.13) mg/dl, NS).

BILE ACID ANALYSIS

High total bile acid concentrations were found in meconium from newborns of the non-UDCA group when compared with the control group of uncomplicated pregnancies (13.5 (5.1) v 2.0 (0.5) $\mu\text{mol/g}$, p<0.01), reflecting the elevated total bile acid concentrations in the maternal serum (figs 1 and 2). Reinforcing the influence of maternal serum bile acid composition on meconium bile acid profiles during ICP, cholic acid concentration was considerably

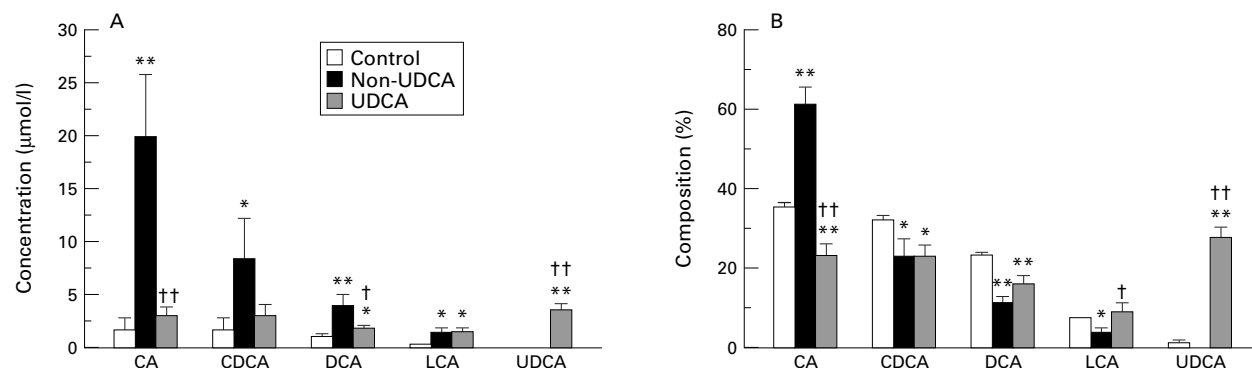


Figure 1 Concentration (A) and percentage composition (B) of the usual bile acids in serum of normal pregnant women (control; n = 8) and patients with cholestasis of pregnancy receiving classical palliative treatment (non-UDCA group; n = 7) or treated with ursodeoxycholic acid (UDCA) until labour (UDCA group; n = 9). Results are expressed as mean (SEM). **p<0.01 and *p<0.05, non-UDCA and UDCA v control; ††p<0.01 and †p<0.05, non-UDCA v UDCA. CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid.

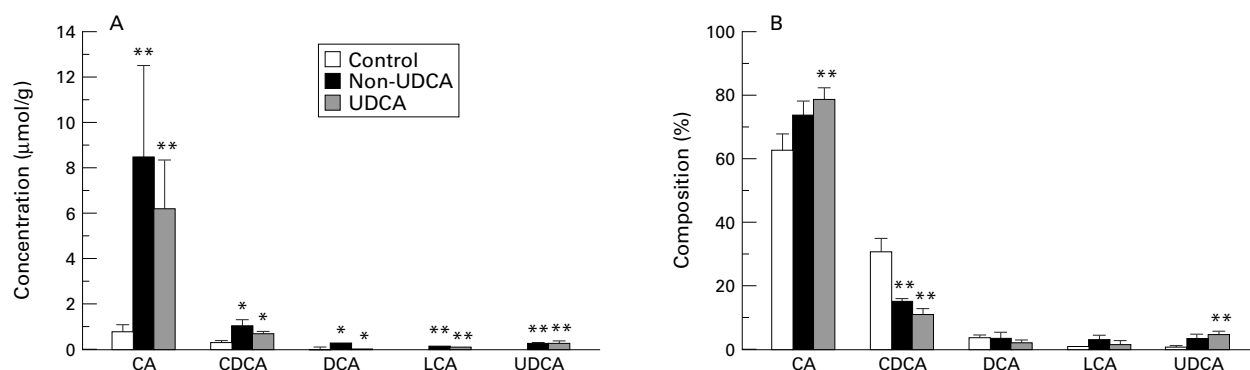


Figure 2 Concentration (A) and percentage composition (B) of the usual bile acids in meconium samples of newborns from normal pregnancies (control; $n = 8$) and from pregnancies complicated by intrahepatic cholestasis of pregnancy receiving classical palliative treatment (non-UDCA group; $n = 7$) or treated with ursodeoxycholic acid (UDCA) until labour (UDCA group; $n = 9$). Results are expressed as mean (SEM). ** $p < 0.01$ and * $p < 0.05$, non-UDCA and UDCA *v* control. CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid.

increased in both the maternal serum (19.8 (6.2) *v* 1.7 (0.1) $\mu\text{mol/l}$; $p < 0.01$) and the meconium (8.4 (4.1) *v* 0.8 (0.3) $\mu\text{mol/g}$; $p < 0.01$). Even though this study was conducted using a limited population, there appears to be a relation between the concentration of total bile acids ($r = 0.85$; $p < 0.01$) and cholic acid ($r = 0.84$; $p < 0.01$) in maternal serum and meconium in ICP, which was not found in the controls. Surprisingly, after UDCA administration, total bile acid and cholic acid concentrations in the meconium (9.2 (2.2) and 6.1 (2.2) $\mu\text{mol/g}$ respectively) were no longer related to those in the maternal serum (17.2 (3.4) and 6.8 (2.6) $\mu\text{mol/l}$). Moreover, treatment did not induce any significant changes in concentration of all the major bile acids in the meconium (fig 2). With regard to bile acid proportions in the meconium, cholic acid was the predominant species in controls and remained as such in both untreated and UDCA treated ICP groups; chenodeoxycholic acid represented about 30% of total bile acids and diminished to less than 20% as a consequence of the disease (fig 2). Despite the significantly lower cholic acid percentage in the maternal serum after UDCA treatment (23.3 (3.4) *v* 61.3 (4.9)%, $p < 0.01$), there was a slightly higher proportion of this bile acid in the meconium (figs 1 and 2). The discrepancy is disclosed by a negative correla-

tion for this variable between the two biological fluids ($r = -0.71$; $p < 0.01$), and furthermore validates the absence of any significant modification in the meconium composition of major bile acids by UDCA administration. Although UDCA percentage in the meconium was slightly increased after treatment compared with the untreated group (5.2 (1.2) *v* 3.9 (1.3)%), it was still much lower than that detected in the maternal serum (28.0 (2.8)%, $p < 0.01$). Also, UDCA concentration in the meconium showed only a 2-fold increase after treatment, despite the much greater increase observed in the maternal serum ($p < 0.01$). Finally, UDCA concentration in the meconium after treatment correlated significantly with the maternal serum concentration of this bile acid ($r = 0.74$; $p < 0.01$), in contrast with that found for the rest of the major bile acids.

Table 2 gives the concentrations of total and individual unusual bile acids in meconium. The data show a significant increase in total unusual bile acids during ICP as compared with the controls. Hyocholic and *allo*-cholic acids represented the bulk of the unusual bile acid species in normal meconium, accounting for 16.7 (2.7) and 4.1 (0.5)% of the total respectively; while *allo*-cholic acid percentage remained unchanged during ICP, hyocholic acid was considerably decreased by the disease (6.5 (0.8)%, $p < 0.01$). Also, 12 α -hydroxy-3-

Table 2 Usual and unusual bile acids in meconium of newborns from patients with intrahepatic cholestasis of pregnancy

	Control	Non-UDCA	UDCA
Usual bile acids ($\mu\text{mol/g}$)			
Total	1.13 (0.34)	9.96 (4.37)*	7.17 (2.11)†
Unusual bile acids ($\mu\text{mol/g}$)			
3 β ,7 α -Dihydroxy-5 β -cholanoic	0.03 (0.01)	0.19 (0.06)*	0.12 (0.04)†
3 α ,6 α -Dihydroxy-5 β -cholanoic (hyodeoxycholic)	0.04 (0.01)	0.22 (0.06)*	0.15 (0.04)†
12 α -Hydroxy-3-oxo-5 β -cholanoic	0.02 (0.01)	0.35 (0.13)*	0.18 (0.04)††
3 α ,7 α ,12 α -Trihydroxy-5 β -homocholanoic	0.01 (0.01)	0.07 (0.02)*	0.05 (0.01)†††
3 α ,6 α ,7 α -Trihydroxy-5 β -cholanoic (hyocholic)	0.30 (0.06)	0.62 (0.18)	0.51 (0.21)
3 α ,7 α ,12 α -Trihydroxy-5 α -cholanoic (<i>allo</i> -cholic)	0.09 (0.03)	0.59 (0.24)	0.29 (0.10)
7 α ,12 α -Dihydroxy-3-oxo-4-cholenoic	0.03 (0.02)	0.16 (0.06)	0.06 (0.01)
3 α -Hydroxy-7-oxo-5 β -cholanoic	0.02 (0.01)	0.14 (0.04)*	0.05 (0.01)†
12 α -Hydroxy-3,5,7-choltrienoic	0.01 (0.01)	0.09 (0.03)*	0.03 (0.01)
2 β ,3 α ,7 α ,12 α -Tetrahydroxy-5 β -cholanoic	0.02 (0.01)	0.08 (0.02)	0.03 (0.01)
3 α ,12 α -Dihydroxy-7-oxo-5 β -cholanoic	0.04 (0.02)	0.16 (0.04)*	0.13 (0.05)
Others	0.25 (0.01)	0.84 (0.29)*	0.44 (0.10)
Total	0.86 (0.21)	3.52 (1.24)*	2.04 (0.57)†
Proportion of unusual bile acids	45.9 (3.4)	32.2 (5.0)	27.2 (6.1)†

Concentrations of total usual and individual unusual bile acids, and proportion of unusual species in meconium of newborns from normal pregnancies (control; $n = 8$), and from pregnancies complicated with intrahepatic cholestasis of pregnancy receiving classic palliative therapy (non-UDCA group; $n = 7$) or treated with ursodeoxycholic acid (UDCA) until labour (UDCA group; $n = 9$). Values are expressed as mean (SEM). * $p < 0.05$, non-UDCA *v* control; † $p < 0.05$ and †† $p < 0.01$, UDCA *v* control.

Table 3 Pattern of bile acid conjugation in meconium of newborns from patients with intrahepatic cholestasis of pregnancy

	Control	Non-UDCA	UDCA
Unconjugated			
CA	2.5 (0.9)	1.7 (0.5)	0.8 (0.3)
CDCA	2.4 (0.9)	0.8 (0.4)	0.3 (0.2)†
DCA	0	0.7 (0.4)	0.3 (0.2)
LCA	0	0	0.1 (0.1)
UDCA	0	0.2 (0.1)*	0.3 (0.2)
Glycine conjugated			
CA	15.4 (2.9)	33.0 (10.4)	36.8 (3.2)††
CDCA	6.7 (0.8)	4.1 (1.6)	2.4 (0.4)††
DCA	2.2 (1.7)	1.5 (0.5)	1.1 (0.1)
LCA	0.2 (0.2)	0.6 (0.3)	0.4 (0.1)
UDCA	0.2 (0.2)	1.0 (0.5)	1.2 (0.3)†
Taurine conjugated			
CA	25.1 (8.7)	19.3 (7.4)	36.7 (8.4)
CDCA	13.5 (2.1)	13.6 (11.1)	5.0 (0.3)††
DCA	3.2 (1.4)	1.4 (1.2)	0.8 (0.2)
LCA	1.3 (0.5)	0.2 (0.1)	0.2 (0.1)
UDCA	2.1 (0.7)	0.4 (0.3)*	0.6 (0.1)†
Sulphated			
CA	3.0 (1.4)	6.7 (4.6)	4.0 (1.1)
CDCA	21.9 (9.4)	12.1 (6.3)	4.5 (2.4)
DCA	0.2 (0.2)	1.5 (0.4)*	0.8 (0.7)
LCA	0	0.6 (0.1)**	0.4 (0.4)
UDCA	0	0.5 (0.1)**	3.5 (2.6)

Proportions of usual bile acids in meconium from normal pregnancies (control; n=3), and from pregnancies complicated with intrahepatic cholestasis of pregnancy receiving classical palliative therapy (non-UDCA group) or treated with ursodeoxycholic acid (UDCA) until labour (UDCA group). Meconium samples from newborns of ICP patients included three from each group (low, medium and high total bile acid concentrations). Values are expressed as mean (SEM). * $p < 0.05$ and ** $p < 0.01$, non-UDCA *v* control; † $p < 0.05$ and †† $p < 0.01$, UDCA *v* control. CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid.

oxo-5 β -cholanoic acid was increased both in concentration and percentage as a consequence of ICP ($p < 0.05$). UDCA treatment did not influence the bile acid pattern in the meconium of newborns of patients with ICP. Nevertheless, a slight decrease was observed for the concentration and percentage of unusual bile acids after UDCA administration.

After separation of unconjugated and conjugated bile acids in the meconium of newborns from patients with ICP by lipophilic anion exchange chromatography, the unconjugated fraction contained only small proportions (<3%) of the total bile acids, irrespective of whether UDCA was given, which was similar to that found in controls (~5%) (table 3). As expected, conjugation occurred mainly with taurine (~45%), followed by glycine (~25%) and sulphate (~25%) groups in meconium samples of newborns from healthy pregnant women. Conversely, in meconium during ICP, an equal representation of glycine and taurine conjugates was noticed in both the non-UDCA (~40 and ~35% respectively) and UDCA (~41 and ~43% respectively) groups. The proportion of sulphate conjugated bile acids in meconium of newborns from patients with ICP (~21%) was similar to controls, but was smaller in the UDCA group (~13%). Sulphated chenodeoxycholic acid was the major species in the sulphate fraction accounting for ~12% of the total bile acids identified in meconium during ICP, and for ~5% after UDCA treatment.

Discussion

Data on meconium bile acid composition in newborns from patients with ICP are scant, and changes that occur on UDCA administration during pregnancy have never been evaluated. In this study, we investigated the bile acid profile and the conjugation pattern in meco-

nium collected from neonates born of healthy women and of patients with ICP. Serum bile acid composition was also analysed at both diagnosis and delivery to determine whether the bile acid concentration in the maternal serum influences the distribution of these compounds in the meconium. Furthermore, as UDCA has been confirmed as the only effective drug for the treatment of ICP during the last trimester and until delivery, we questioned whether this bile acid reverses alterations in the maternal serum and meconium that the disease may cause. Finally, because concerns have been raised about the toxicity of lithocholic acid or other metabolites of UDCA, we also quantified these species in the meconium.

Maternal intrahepatic cholestasis results in considerable increases in both total bile acid (6-fold) and cholic acid (12-fold) concentration in the serum. Similar alterations in meconium samples of newborns of patients with ICP suggest that these are the result of placental transfer of bile acids from mother to fetus. As the fetal colon is sterile, the presence of products of bacterial metabolism such as deoxycholic and lithocholic acids in increased levels provides further support for the hypothesis that some of the bile acids found in the meconium of these neonates are of maternal origin and reach the fetus by transfer across the placenta.^{25 45}

Improvement of pruritus and cholestasis in the mother,¹³⁻²¹ as well as a consequent decrease in the bile acid pool in the fetus, have been described as beneficial effects of UDCA treatment. Increasing evidence suggests that UDCA reduces bile acid levels in colostrum,⁴⁶ serum cord blood,²² and amniotic fluid.²³ Interestingly, UDCA administration appears not to influence concentrations of the major bile acids in meconium including that of its toxic metabolite, lithocholic acid, despite the small increase in its percentage detected in maternal serum ($p < 0.05$). UDCA concentration in the meconium is determined by its increase in the maternal serum, but exhibited only a 2-fold increase after treatment, whereas a larger increase was observed in maternal serum. Most striking was the observation that cholic acid percentage significantly decreased in the mother but slightly increased in the fetus. This suggests an accumulation of cholic acid in the meconium, which is retained in the fetal compartment and consequently cannot be modified by treatment. Aggravating this situation is the continued ingestion by the fetus of amniotic fluid which is also enriched in cholic acid.^{9 11} The lack of effect of UDCA treatment on bile acid concentration in the meconium is not in disagreement with the improved placental transport of bile acids¹² or the decreased concentrations of total bile acids and cholic acid found in other fetal biological fluids^{22 23} after UDCA administration. On the contrary, the lack of ability of the fetus to excrete meconium (in normal conditions) and the reduced intestinal absorption are responsible for accumulation of bile acids in the meconium which cannot be modified by the

treatment. In this sense, UDCA treatment should be initiated as soon as the diagnosis of ICP is made, mainly to decrease bile acid levels in the maternal serum and therefore restrict the accumulation of these compounds in the meconium.

As bile acid metabolism in the newborn infant differs from that in the adult, it is not unexpected that a variety of unusual bile acids should be present in normal meconium, resembling the composition of fetal gallbladder bile.^{26,27} In addition to cholic, chenodeoxycholic, deoxycholic, and lithocholic acids, significant amounts of hyocholic, hyodeoxycholic, *allo*-cholic, and *7 α ,12 α* -dihydroxy-3-oxo-4-cholenoic acids were also identified during this work, which is in accordance with the results of others.^{24,28,47-49} Their increased levels in meconium during ICP are probably due to the ingestion of amniotic fluid containing increased amounts of bile acids as a consequence of cholestasis. Moreover, the presence of unusual bile acids may also reflect hepatic immaturity and/or activation of liver enzymes that are not important later in life.

In agreement with our data, previous studies have shown that *6 α* -hydroxylation of bile acids is a major metabolic reaction during the prenatal period^{24,50} and therefore hyocholic acid is the predominant unusual bile acid found in meconium. In this study, proportions of hyocholic (17%) and chenodeoxycholic (31%) acids in normal meconium were similar to those (19% and 29% respectively) reported previously.^{28,47} Interestingly, the significant decrease in chenodeoxycholic acid percentage in meconium during ICP was paralleled by a similar decrease in hyocholic acid proportion. These changes were not influenced by UDCA treatment.

Although bile acid conjugation in normal meconium was more pronounced with taurine than with glycine, in agreement with the results of others,^{50,51} the predominance of glycine conjugates during ICP found in this study may result from restricted maternal to fetus transport across the placenta for taurine conjugates. With regard to the sulphate conjugated fraction, it has been shown to represent a large proportion of the total bile acids in meconium of newborns from both uncomplicated pregnancies and ICP.⁵² These findings were confirmed in this study, in which sulphated bile acids accounted for ~21% of the total in meconium of neonates from untreated ICP, but were only ~13% in neonates from UDCA treated ICP. Whether a decrease in sulphation, considered by some to be an important pathway to the detoxification of hepatotoxic bile acids, is due to the effect of UDCA in the fetus is still to be investigated.

In conclusion, increased bile acid levels in maternal serum during ICP induce a considerable increase in these compounds in the meconium, with cholic acid representing the major species. UDCA administered to the mother until labour corrects the bile acid profile in the maternal serum but does not influence the meconium bile acid pattern of ICP. Moreover,

this beneficial treatment for the mother does not increase meconium levels of potentially toxic metabolites of UDCA such as lithocholic acid.

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