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Characteristics of bile acids metabolism profile in the second and third trimesters of normal pregnancy

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ABSTRACT

Purpose: Bile acids are a group of cholesterol metabolites functioning as key regulators of glucose, lipid, and energy metabolism. Their homeostatic control is essential to the physiology of the normal pregnancy. Abnormalities of bile acids regulation in pregnancy lead to intrahepatic cholestasis of pregnancy, a serious condition associated with a number of fetal and maternal morbidities. Dysregulation of glucose and lipids is also tied to perturbations in bile acid concentrations. Changes in bile acid metabolic profiles in the second and third trimesters of pregnancy have been incompletely explored. We seek to establish pregnancy-specific normative ranges for a number of bile acids in women in the second and third trimesters and explore changes in their concentrations in the period from 12 to 40 weeks gestation.

Procedure: In this cross-sectional study, a total of 782 normal pregnant women were enrolled including n = 290 in the second trimester (12–28 weeks) and n = 492 in the third trimester (29–40 weeks). The concentrations of 14 bile acids were measured by liquid chromatography and mass spectrometry (LC-MS) and compared at various time points. Reference intervals of these bile acids were calculated using standard statistical techniques.

Results: A reference interval profile of 14 bile acids from a cohort of 782 normal pregnant women was developed. Significant differences in concentration were found between the second trimester and the third trimester. Unconjugated bile acids dominate the bile acid profile in the second trimester, while conjugated bile acids, especially (taurine-conjugated) dominate in the third trimester. 28–31 weeks gestation was the notable change period of bile acid metabolism.

Conclusion: This study establishes pregnancy-specific reference intervals for bile acids in the second and third trimesters. As bile acid composition changes with gestational age, this study establishes a foundation for trimester-specific clinical interpretation of bile acid metabolic profiles in pregnant women.

1. Introduction

Bile acids are a group of cholesterol metabolites representing the major chemical component of bile. They undergo synthesis, transmembrane transport, and enterohepatic circulation permitting micellization and absorption of dietary fats and the fat soluble vitamins. They also serve as signaling molecules, controlling metabolism and inflammation [1]. Primary bile acids are synthesized from cholesterol in two pathways within the liver: the classical pathway regulated by CYP7A1 to produce cholic acid (CA), the alternative pathway mainly controlled by CYP27A1 to produce chenodeoxycholic acid (CDCA). The cytotoxicity of CA and CDCA are reduced by conjugation to glycine and taurine increasing water solubility and facilitating transmembrane transport [2,3]. Primary bile acids enter the intestinal tract and are converted to secondary bile acids including lithocholic acid (LCA), deoxycholic acid (DCA) and ursodeoxycholic acid (UDCA) by intestinal microbiota [4,5]. Some 95% of bile acids are reabsorbed into the portal vein via the small intestine. Hepatocytes uptake 99% of the reabsorbed bile acids through a Na^+-taurocholate co-transporting polypeptide (NTCP) or organic anion transporting polypeptide (OATPs) with the remaining 1% entering the peripheral circulation [6].

Concentration of bile acids in plasma is strictly regulated by a complex system of membrane and nuclear receptors in the liver and intestine, including bile salt export pump (BSEP), nuclear farnesoid X receptor (FXR), G-protein-coupled bile acids receptor 1 (GPBAR1), multidrug resistance p-glycoprotein 3 (MDR3), and NTCP as discussed elsewhere [7]. BSEP is the major transporter of bile acids to the bile

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canaliculi and is considered to be a key factor in the clearance of bile acids from plasma [8]. FXR, mainly expressed in hepatocytes and enterocytes, acts as a bile acid receptor and is principally activated by primary bile acids. Activation of FXR upregulates the expression of a small heterodimer partner (SHP) protein which inhibits CYP7A1 and NTCP expression and both bile acid synthesis and uptake [9]. FXR also induces the expression of BSEP and MDR3, promoting the excretion of bile acids into the bile canaliculi which activates the secretion of fibroblast growth factor 19 (FGF19) in the distal ileum. This in turn feeds back to the liver to activate tyrosine kinase receptor FGF receptor 4 and βKlotho (a single transmembrane protein), ultimately inhibiting CYP7A1 transcription [10,11].

The FXR and GPBAR1 proteins of the bile acid pathway also participate in glucose homeostasis. Ileal enteroendocrine membrane receptor GPBAR1 is differentially activated by LCA > DCA > CDCA > CA which leads to Glucagon-like peptide-1 (GLP1) secretion. The GLP1 is transported to the pancreas via the portal vein promoting insulin secretion from islet β cells to control plasma glucose [12–14]. FXR knockout leads to insulin resistance and impaired glucose tolerance confirming that bile acids influence glucose regulation via FXR [15]. It has also been shown that FXR is differentially activated by CDCA > DCA > LCA > CA implying that differences in bile acids profiles will affect glucose homeostasis [13].

Pregnancy is a unique physiological state wherein physiology changes dramatically compared to the nonpregnant state. Pursuant to the present discussion, in pregnancy there are increases in plasma lipids, steroid hormones, binding globulins, and numerous metabolic changes to cope with fetal nutritional demands [16]. In most pregnant women, there is a modest increase in bile acid concentrations. It has been demonstrated that estrogen, progesterone and their metabolites affect bile acids metabolism in pregnancy. Specifically, estrogen and its metabolites are capable of inhibiting FXR, BSEP, and NTCP expression and can also promote CYP7A1 activity which may lead to excessive bile production and cholestasis [17,18]. Progesterone metabolite PM5, acting as an FXR agonist, interferes with normal bile acid homeostasis causing a gestationally high bile acid phenotype [19]. PM4S, another metabolite of progesterone, also inhibits BSEP-mediated bile acid excretion [20]. The overall biological effects of estrogen, progesterone and their metabolites in pregnancy are to both increase bile acid production and reduce bile canaliculi discharge, leading to elevation of total bile acid (TBA) concentration in the peripheral blood of pregnant women relative to the non-pregnant state.

In recent decades, it has been observed that bile acids, acting as key metabolic regulators, are associated with pregnancy-related diseases in the second and third trimesters, including intrahepatic cholestasis of pregnancy (ICP), gestational diabetes mellitus (GDM), and gestational hyperlipidemia. A diagnosis of ICP can be made when pregnant women present with pruritus, abnormal liver function tests and increases in bile acid concentrations. Increases in bile acids may also be associated with insulin resistance and the development of GDM [21,22]. Additionally, bile acids directly induce metabolic derangement of lipids leading to pregnancy-related hyperlipidemia. For these reasons, normal ranges for bile acids and their metabolites are required for the identification of the pregnancy-related bile acid disorders and for the elucidation of the underlying pathophysiology.

The purpose of this study was to establish pregnancy-specific normal ranges for 14 bile acids in the second and third trimesters (12–40 weeks of gestation) (n = 782), covering a larger range of reproductive ages (18–45 years). These results provide a reference point for the investigation and characterization of pregnancy-related diseases affected by bile acid metabolism.

2. Materials and Methods

2.1. Study Population and Sample Collection

Clinical data were extracted from the medical records of patients in Women's Hospital, School of Medicine, Zhejiang University, China. Between August 2016 to February 2017, 782 normal individuals were enrolled in this study. Individuals enrolled were aged 18–45 years. Subjects at high risk for bile acid abnormality were excluded from participation. Exclusions included: multiple pregnancy, in vitro fertilization-embryo transfer (IVF-ET), personal or family history of ICP, and personal history of liver disease. Representative demographic and laboratory data are shown in supplemental Table 1. This study was approved by the human subjects institutional review board of Women's Hospital, School of Medicine, Zhejiang University. The samples were obtained from all participants after 8–14 h of fasting. Sample transfer, centrifugation, and separation were completed within 1 h to avoid any preanalytical factors that may affect bile acid concentrations. Samples were stored at −80 °C until analysis.

2.2. Bile Acid Measurement

Chemicals: Reference standards of cholic acid (CA), deoxycholic acid (DCA), glycocholic acid (GCA), glycoursodeoxycholic acid (GUDCA), glycochenodeoxycholic acid (GCDCA), taurodeoxycholic acid (TDCA), taurocholic acid (TCA), and ursodeoxycholic acid (UDCA) were purchased from Toronto Research Chemicals Inc. (Toronto, Canada). Chenodeoxycholic acid (CDCA), lithocholic acid (LCA), glycodeoxycholic acid (GDCA), tauroursodeoxycholic acid (TUDCA), taurochenodeoxycholic acid (TCDCA), and taurolithocholic acid (TLCA) were purchased from Sigma-Aldrich (St. Louis, USA). HPLC-grade methanol, formic acid, and ammonium acetate were obtained from ANPEL Laboratory Technologies (Shanghai, China). HPLC-grade acetonitrile was from Merck (Darmstadt, Germany).

Sample preparation: an aliquot of 100 μl of serum was mixed with 200 μl of acetonitrile and 10 μl of methanol with an internal standard (IS) solution. The IS included: CA-D4 (1 μg/ml), UDCA-D4 (5 μg/ml), CDCA-D4 (5 μg/ml), DCA-D5 (5 μg/ml), GCA-D5 (5 μg/ml), GCDCA-D5 (5 μg/ml), and TCDCA-D5 (2 μg/ml). The mixture was then vortexed for 2 min and then centrifuged at 13000 rpm at 4 °C for 15 min. To the 100 μl of supernatant, 100 μl of the initial HPLC solvent gradient mixture was added, and the mixture was then vortexed for 30 s. The supernatant was used for LC-MS analysis.

LC-MS analysis: Bile acids analysis was performed by ultra-high performance liquid chromatography (UHPLC, Agilent Technologies, Santa Clara, USA) coupled to an API 5500 triple quadrupole tandem mass spectrometer (SCIEX, Framingham, USA). All chromatographic separations were performed with a Poroshell 120 EC-C18 column (1.7 μm, 3 mm × 50 mm) (Agilent Technologies, Santa Clara, USA).

The mobile phase consisted of 10 mM ammonium acetate in LC-MS grade water (mobile phase A) and 0.1 formic acid acetate in LC-MS grade acetonitrile (mobile phase B) run at a rate of 0.3 ml/min. The flow gradient was as follows: 0 (65% A), 0–3 min (60% A), 3–8.5 min (0% A), 8.5–8.6 min (65% A), 8.7–10 min (65% A). The column was maintained at 35 °C, and the injection volume of all samples was 5 μl. The electrospray ionization (ESI) source operated in negative ion mode (ESI−) with a 4.5 kV capillary voltage. The temperature was 550 °C. The Curtain Gas and Collision Gas were 30 V and 9 V, respectively. The Ion Source Gas 1 and Ion Source Gas 2 were 50 V and 55 V, respectively. Data were collected with multiple reaction monitoring (MRM). The DP, CXP, and collision energy for each bile acid are listed in supplemental Table 2.

2.3. Data Analysis

Analyst software v1.6.0 (SCIEX, Framingham, USA) was used for the analysis of mass spectrometric data. Independent samples *t*-test or Mann-Whitney test was used for between group comparisons, in cases of two groups of continuous variables. One-way analysis of variance (ANOVA) or Kruskal-Wallis test was used in cases of more than two groups of continuous variables. A multivariate data analysis of partial least squares was performed using SIMCA-P (version 13.0, Umetrics, Sweden). Bile acid components and clinical data were set as X (independent) and Y (dependent) variables respectively. Raw data was used in

the partial least squares (PLS) analysis. Bile acid data of participants in each gestational age were averaged then normalized for generation of a heat map. Because bile acid distributions were universally skewed, reference intervals were determined nonparametrically and correspond to the 2.5th–97.5th percentiles of the experimental distribution. The statistical analysis was performed on the IBM SPSS statistics 18.0 software and GraphPad Prism 7.0. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Clinical Data of Normal Pregnant Women and Their Correlation with Bile Acid Metabolism

The average age of participants was 31.59 ± 4.48 years, gestational age ranged from 12 to 40 weeks, and average gestational age was 30.06 ± 5.81 weeks. The average results of blood and biochemical data, white blood cell count (WBC), red cell count (RBC), hemoglobin (HGB), platelet count (PLT), total bilirubin (TBIL), indirect bilirubin (IBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (CREA), glucose (GLU), total bile acids (TBA), triglycerides (TG), total cholesterol (CHOL), high density lipoprotein cholesterol (HDL), and low density lipoprotein cholesterol (LDL) were all within the reference range.

Bile acid assays were performed simultaneously on a single sample from each participant using LC-MS. The TBA concentration detected by LC-MS was in agreement with the results detected by enzymatic cycling assay (TBA-clin) (Fig. 1A) in the overall cohort. The average percentage of bile acids components in TBA was shown in Fig. 1B. The PLS analysis of the participants' clinical data and 14 bile acid components was shown in Fig. 1C-a, the overlapped data is enlarged in Fig. 1C-b. Among the clinical data, the gestational age and TBA-clin were the most relevant factors with respect to bile acid metabolism. Additionally, red blood cell count and hemoglobin were also associated with bile acid concentrations.

If the patient age >35 years, pregnancy is considered a high risk of fetal and maternal morbidities. To clarify whether the age influences bile acid profiles, we divided the participants into two age groups, ≤35 y and >35 y. No difference in bile acids metabolism between the two groups was identified (Fig. 1D). In order to investigate the influence of gestational age on TBA (sum of 14 bile acids detected by LC-MS) concentration, we divided the population into second and third trimesters groups. No obvious difference in TBA concentration was identified. We found no significant correlation between the clinical data in the second trimester and the metabolism of TBA. In the third trimester, both red blood cell count and hemoglobin were associated with elevated TBA (Table 1).

3.2. Reference Intervals for 14 Bile Acids and TBA in the Second and Third Trimesters

Because the TBA concentration was associated with neither patient age nor gestational age, we analyzed the changes of TBA and bile acid components and based on our findings, built bile acid component reference intervals for the second and third trimesters. Interestingly, though the TBA concentration was stable and within the range of 1.9 ± 0.04 μM, bile acid profiles altered within individuals as the gestation progressed (supplemental Table 3). Specifically, the ratio of CA/CDCA in the second trimester was 1.4 ± 0.12 compared with the 1.6 ± 0.05 in the third

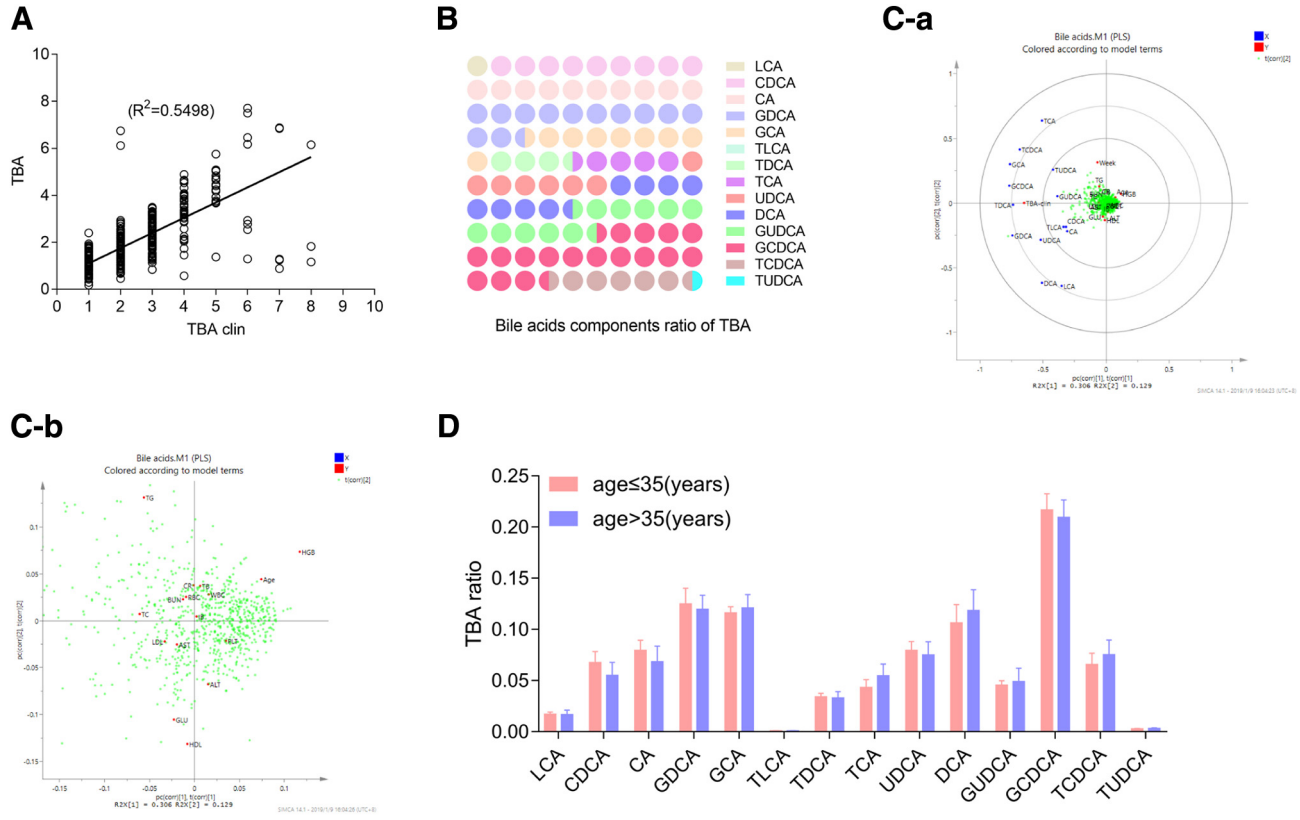

Fig. 1. Correlation of the total bile acids concentration measured by LC-MS and enzymatic cycling assay (1A), percentage of 14 components in total bile acids (1B), PLS analysis of 14 bile acids components and clinical data in 782 normal pregnant participants. The green squares represent all included individuals, red squares represent various clinical data, and black triangles represent bile acids components. Abbreviations: CA, cholic acid; DCA, deoxycholic acid; GCA glycocholic acid; GUDCA, glycoursodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; TDCA taurodeoxycholic acid; TCA, taurocholic acid; GLCA, glycolithocholic acid; UDCA, ursodeoxycholic acid; CDCA, chenodeoxycholic acid; LCA, lithocholic acid, GDCA, glycodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; TCDCA, taurochenodeoxycholic acid, TLCA taurolithocholic acid; TBA clin, total bile acids measured by enzymatic cycling assay (1C-a and 1C-b). Bile acids components keep stable in high and low risk groups (1D).

Table 1
Characteristics of participants with low and high TBA concentrations in the second and third trimesters and the statistical analysis.

Variables	Second trimester (n = 290)			Third trimester (n = 492)		
	Low TBA (n = 145)	High TBA (n = 145)	*P* value	Low TBA (n = 246)	High TBA (n = 246)	*P* value
Age, median (range), years	31.48 ± 4.45	31.23 ± 4.74	0.4666	31.84 ± 4.46	31.42 ± 4.48	0.2926
Gestational age, median (range), week	23.9 ± 3.01	23.68 ± 2.76	0.2536	33.64 ± 3.30	33.86 ± 3.62	0.5731
WBC, median (range), ×10^9/L	9.63 ± 2.14	9.94 ± 2.36	0.3217	9.413 ± 2.42	9.24 ± 2.11	0.5309
RBC, median (range), ×10^9/L	3.79 ± 0.34	3.71 ± 0.33	0.0612	3.93 ± 0.32	3.87 ± 0.36	0.0277
HGB, median (range), g/L	116.4 ± 7.92	115.3 ± 8.94	0.241	119.7 ± 9.48	116.8 ± 10.27	0.0003
PLT, median (range), ×10^9/L	220.8 ± 47.93	216.6 ± 49.49	0.5405	202.2 ± 52.71	196.2 ± 44.53	0.173
TBIL, median (range), μmol/L	7.07 ± 2.71	6.66 ± 2.34	0.2672	7.791 ± 3.07	7.633 ± 2.79	0.9833
IBIL, median (range), μmol/L	4.58 ± 1.96	4.31 ± 1.75	0.3275	5.10 ± 2.23	5.05 ± 2.10	0.7683
ALT, median (range), U/L	19.05 ± 10.28	18.94 ± 8.77	0.5569	13.65 ± 5.53	13.89 ± 6.36	0.9698
AST, median (range), U/L	19.6 ± 6.06	20.19 ± 5.75	0.3739	18.89 ± 5.35	19.4 ± 5.83	0.4298
BUN, median (range), mmol/L	3.01 ± 0.70	3.05 ± 0.66	0.5873	3.07 ± 0.76	3.078 ± 0.86	0.6982
CREA, median (range), μmol/L	65.42 ± 5.36	65.26 ± 4.87	0.9749	66.91 ± 6.54	66.77 ± 6.00	0.9588
GLU, median (range), mmol/L	4.48 ± 0.30	4.48 ± 0.35	0.5936	4.44 ± 0.37	4.47 ± 0.39	0.6538
TG, median (range), mmol/L	2.03 ± 0.67	2.00 ± 0.69	0.4881	2.68 ± 0.94	2.76 ± 1.01	0.6952
CHOL, median (range), mmol/L	6.24 ± 1.032	6.01 ± 0.90	0.0597	6.12 ± 1.06	6.25 ± 1.14	0.2929
HDL, median (range), mmol/L	1.94 ± 0.40	1.91 ± 0.35	0.4296	1.78 ± 0.38	1.76 ± 0.35	0.5268
LDL, median (range), mmol/L	2.89 ± 0.81	2.75 ± 0.71	0.1119	2.76 ± 0.75	2.8 ± 0.77	0.4381

Table 2
Distribution of non-significant changed serum bile acids concentrations in the second and third trimesters.

Bile acids (nM)	Mean (n = 782)	Median (n = 782)	2.5%–97.5%
CDCA	125	75	10–546
CA	148	73	20–718
GDCA	253	185	2–897
TLCA	2	1	0.4–5
UDCA	142	113	17–466
DCA	195	153	11–598
GUDCA	98	50	9–474
GCDCA	433	338	69–1448
TUDCA	6	3	0.4–31

trimester ($P = 0.07$), indicating that no significant change in the flux through classic and alternative synthesis pathways occurred. Reference ranges of bile acids showing no significant change between the second and third trimester are shown in Table 2. Certain bile acids did show significant change with gestational age and are presented by trimester in Table 3. All of GCA, TCA, TCDCA, TDCA increased significantly in the third trimester, while the glycine conjugated/taurine conjugated and LCA decreased. Total conjugated bile acids/total unconjugated bile acids also showed obvious increases in the third trimester relative to the second.

3.3. Characteristic Changes in Weekly Bile Acid Profiles

For a more detailed view of changes in bile acid profiles in the second and third trimesters, TBA and bile acid components concentrations were measured weekly among the participants. A heat map revealed the dynamic changes of bile acid components with each gestational week specifically (Fig. 2A). Note that subjects in the 12–18 week range were excluded from this analysis due to a paucity of participants. The overall analysis indicated that the relative concentration of unconjugated (CA, CDCA, UDCA, LCA, DCA) and conjugated bile acid (TCA, GCA, TCDCA, GCDCA, TDCA, GDCA, TLCA, TUDCA, GUDCA) flip sometime in the range of 28–31 weeks. Unconjugated bile acids are the dominant contributors to the TBA concentration prior to 28–31 weeks, while conjugated bile acids eventually represent the majority of TBA as the pregnancy proceeds (Fig. 2B and C).

Table 3
Distribution of significant changed serum bile acids concentrations in the second and third trimesters.

Bile acids (nM)	Second trimester (n = 290)			Third trimester (n = 492)		
	Mean	Median	2.5%–97.5%	Mean	Median	2.5%–97.5%
LCA	31	25	3–99	24	20	3–68
GCA	188	158	43–512	242	187	47–743
TCA	55	43	8–182	93	64	13–369
TDCA	57	39	0.4–235	68	50	0.4–225
TCDCA	112	87	20–366	132	91	19–515
Total con/Total uncon	10	8	3–36	12	10	4–36
G/T	5	4	1.3–13	5	4	0.9–14

3.4. Conjugated and Unconjugated Bile Acid Components Change Prior to and After 28–31 Weeks

Among all the conjugated and unconjugated bile acids, 7 components, including UDCA, DCA, LCA, TDCA, GCA, TCA, TCDCA, changed significantly during the 19–40 weeks with respective *P*-values of 0.0163, 0.0034, 0.0003, 0.0004, <0.0001, <0.0001, <0.0001 (supplemental Table 4). The components with coefficients of determination $R^2 > 0.5$ in linear regression analysis are shown in Fig. 3A. LCA, DCA, UDCA decreased with gestational age (Fig. 3B), whereas the other 4 components increased. The remaining 8 components showed no statistically significant change (Fig. 3C). Six bile acids, including UDCA, DCA, LCA, GCA, TCA, TCDCA, changed significantly prior to and after 28–31 weeks gestation: GCA, TCA, TCDCA increased (Fig. 3D-F), and UDCA, DCA, LCA decreased (Fig. 3G-I).

4. Discussion

In this cross-sectional investigation, the metabolic profiles of 14 bile acids in a normal pregnant cohort were studied. PLS analysis showed that gestational age was the most important determinant of bile acid concentrations. The serum TBA concentration remained stable, consistent with previous findings [23,24].

However, by examining a panel of 14 bile acids we have found that concentrations of unconjugated and conjugated bile acids change between the second and the third trimesters, particularly between 28 and 31 weeks, in this Chinese cohort. Unconjugated bile acids (CA, CDCA, LCA, DCA) dominate before 28 weeks while conjugated bile acids concentration increase after 31 weeks. Although there was an inconsistency in the bile acid metabolic profiles between 22 and 24 weeks gestational age compared with 19–21 and 25–27 weeks, no statistical significance observed (data not shown).

The ratio of CA/CDCA did not change significantly with gestational age, indicating the strict regulation of the classical and alternative pathways. However, the total conjugated bile acids and total unconjugated bile acids were seen to rise. In the conjugated forms, ratio of taurine

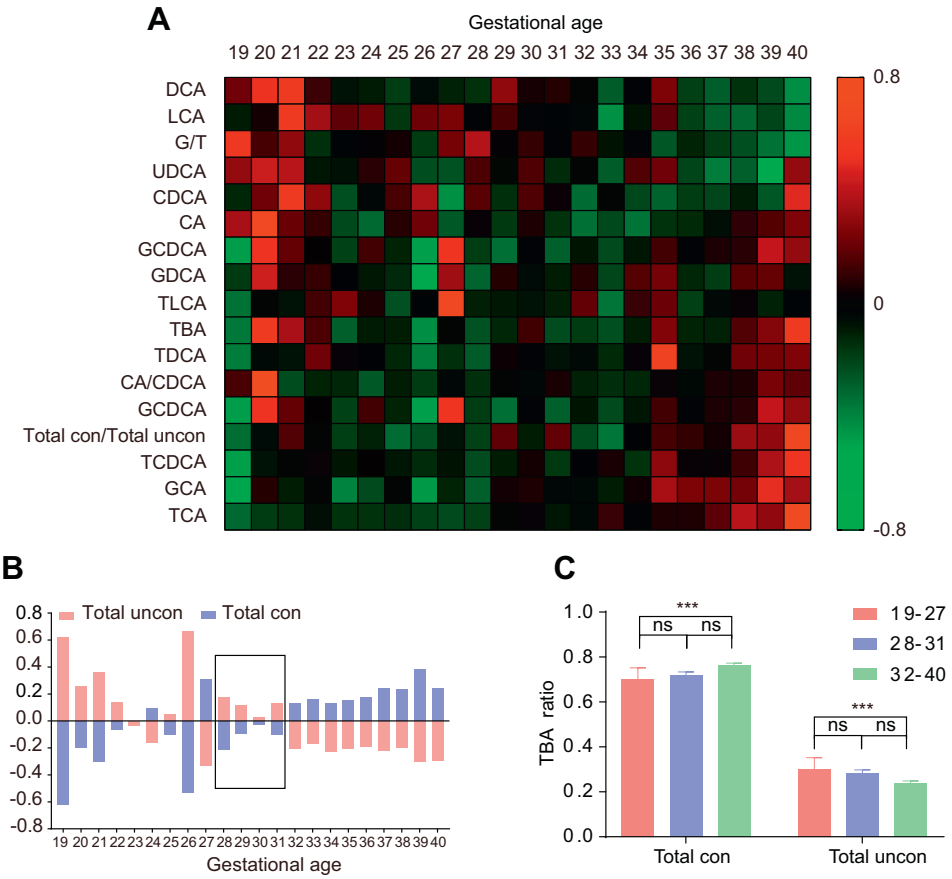

Fig. 2. Heat map of 14 bile acids components and TBA changes in the second and third trimesters (2A), total unconjugated bile acids and total conjugated bile acid metabolism changes with gestational age. Black squares represent the transition period of 28–31 weeks (2B, 2C).

conjugated/glycine conjugated clearly increased as the pregnancy progressed. These changes were likely related to changes in energy conversion and gut microbiota associated with normal physiology of pregnancy. Bile acid metabolites have different solubility, which in turn affects the absorption of nutrients supporting fetal development and maternal lipolysis in the second and third trimesters [25]. Many researchers have observed that the composition of the maternal gut microbiome alters with the progress of gestation [26,27]. Importantly, bile salt hydrolase produced by bacteria in the gastrointestinal tract exhibits higher substrate specificity for glycine conjugated over taurine conjugated bile acids. This likely explains why the ratio of taurine conjugated/glycine conjugated bile acids was observed to increase with gestational age, although the specific bacterial genera regulating individual bile acids was not identified [28,29]. Unfortunately, we did not perform any investigations of the microbiome in this study, which is a limitation.

The homeostatic concentration of TBA throughout the second and third trimesters masked the changes in concentration among the individual bile acids observed during pregnancy. In the study of bile acids induced disorders, we propose that changes of individual bile acids should be considered because the specific composition of bile acids varies with the gestational age. For example, in GDM research, patients are traditionally enrolled at 24–28 weeks gestational because of the stable plasma glucose observed during this period [30]. According to our research, we suggest that in addition to the gestational age, LCA should also be considered when recruiting subjects not only because LCA is involved both in the GBPAR1-GLP1-insulin pathway and FXR-regulated glucose metabolism, but also because LCA concentration declines significantly between the second and third trimesters ($P < 0.001$) [12–14]. Because GDM patients are also characterized by poor insulin sensitivity accompanied with the expected lipid profile of low HDL and high triglycerides, bile acid profiles in lipid metabolism studies may provide new insight into GDM [31]. Additionally, unconjugated and conjugated bile acids exert different effects on fats and fat-soluble vitamin metabolism. For example, in the pregnancy-related vitamin D research, LCA was noted to affect the absorption of vitamin D by activating intestinal vitamin D receptor [32,33]. There may also important effects of bile acids on lipid metabolism in pregnancy related to our observation that the ratio of total conjugated bile acids to total unconjugated bile acids changes over the course of the pregnancy. Unconjugated bile acids, which are helpful in fat digestion and absorption, dominate in the second trimester. Obviously, we are also interested in how bile acid partitioning between unconjugated and conjugated forms affects ICP. Traditionally, TBA-clin and clinical presentation form the diagnostic criteria for ICP, but there is still debate as to the appropriate gestational age division between early and late onset ICP which lead to different outcomes [34,35]. Considering that we saw transition from predominantly unconjugated bile acids to conjugated bile acids as the pregnancy progressed, we are interested to determine if bile acid profiling can help distinguish the early and late onset forms of ICP.

In conclusion, we have established reference intervals for 14 bile acids by gestational age. Bile acids components may vary characteristically in different related disease states and there is a paucity of information on how bile acid profiles may assist in the diagnosis of pregnancy-related disease states showing bile acid derangements. Our study provides a reference point for further study of ICP, GDM, pregnancy-induced hyperlipemia and other bile acids related disorders.

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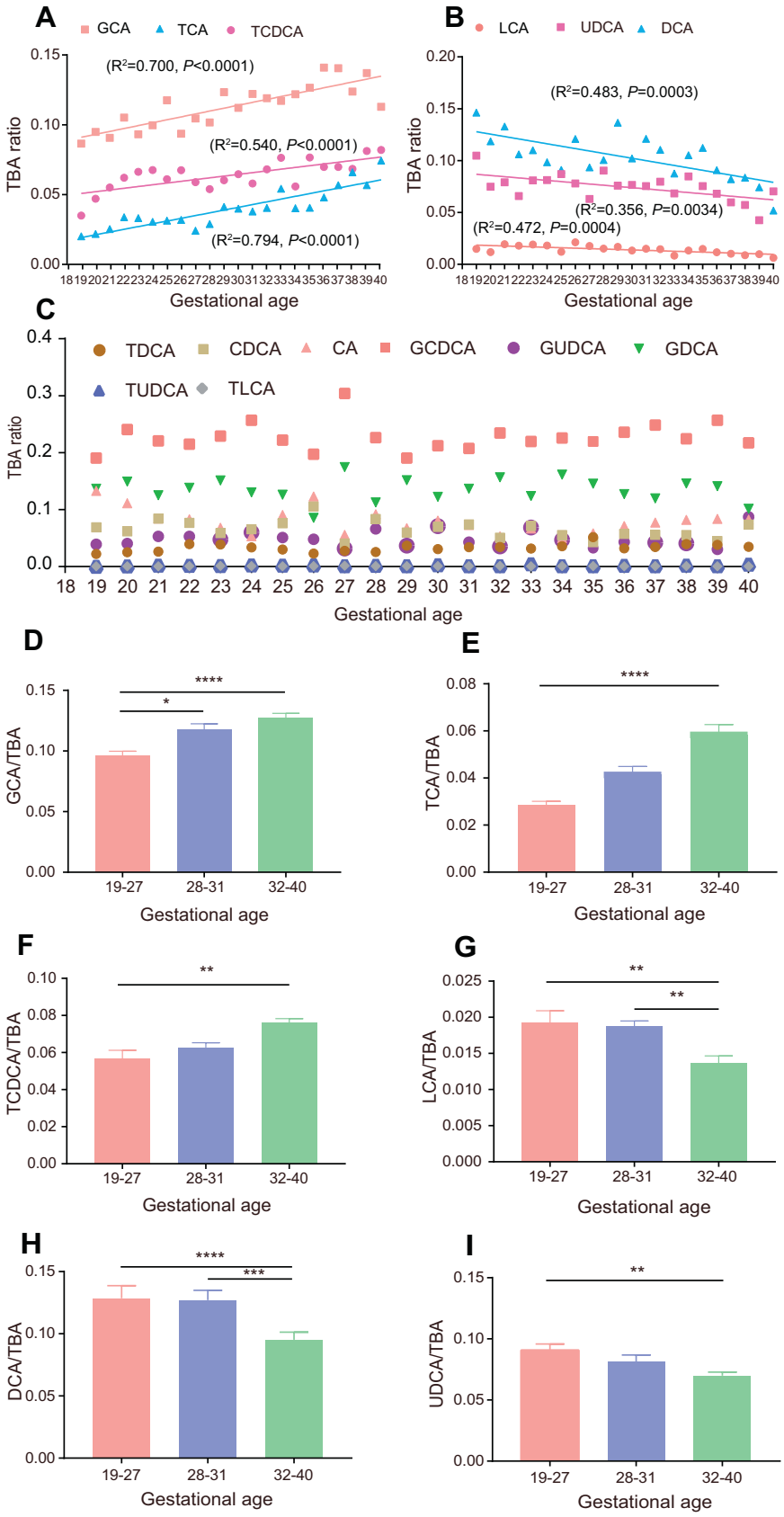

Fig. 3. Linear changes of 14 bile acids components metabolic profiles from 19 to 41 weeks. Data are presented as the ratio to TBA. GCA, TCA, TCDCA changed with $R^2 > 0.5$ (3A). LCA, DCA, UDCA decreased with gestation progressive (3B). There is no obvious change in the remaining 8 components (3C). LCA, DCA, UDCA, TCA, GCA, TCDCA changed significantly prior to and the transition period of 28–31 weeks (3D–I).

Conflict of Interest

None of the authors has any conflict in relation to the study.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2019.04.004.

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