Received: 17 August 2020 | Revised: 8 December 2020 | Accepted: 11 January 2021

DOI: 10.1002/hsr2.243

RESEARCH ARTICLE

Health Science Reports Open Access WILEY

Bile acids during pregnancy: Trimester variations and associations with glucose homeostasis

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Funding information
Danone Institute of Canada; Fondation CHU de Québec; Fonds de Recherche du Québec - Santé

Abstract

Background and aims: Bile acids are known to contribute to hepatic glucose and lipid metabolism regulation. Although glucose homeostasis sustains well-characterized modifications during uncomplicated pregnancies, changes in bile acids concentrations and relative proportions throughout pregnancy remain unknown. Furthermore, literature shows strong associations between bile acids profiles and glucose homeostasis under normal metabolic conditions. We seek, first, to characterize bile acids' metabolic changes across trimesters and, second, to evaluate associations between changes in bile acids and glucose homeostasis indexes in the first and second trimesters.

Methods: A total of 78 women were recruited and followed at each trimester of pregnancy. Fasting serum samples were collected once per trimester in which quantitative measurement of 30 different bile acids' molecules were performed using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Glucose homeostasis indexes were measured in the first and second trimesters, after a 12-hour fast and following a 75 g oral glucose tolerance test.

Results: Total bile acids increased from the first trimester to late pregnancy, along with the cholic acid: chenodeoxycholic acid and conjugated: unconjugated bile acids ratios. Changes in bile acids were positively associated with elevated peripheral and hepatic insulin resistance indexes, as well as with trimestral changes in these indexes.

Abbreviations: BA, bile acids; BMI, body mass index; CA, cholic acid; CDCA, chenodeoxycholic acid; FXR, Farnesoid X receptor; GLP-1, glucagon-like peptide-1; HOMA-IR, homeostasis model assessment of insulin resistance; iAUC C-peptide, incremental area under the curve for C-peptide; ICP, intrahepatic cholestasis of pregnancy; TGR5, G-protein-coupled bile acid receptor.

Conclusion: Our findings suggest that modifications occurring in bile acids' profiles during normal pregnancy are associated with changes in glucose homeostasis. Further research is needed to examine the nature of those associations and the possible outcome of bile acids changes on pathological glucose homeostasis alterations during pregnancy.

KEYWORDS
bile acids, cholestasis, gestational diabetes mellitus, glucose homeostasis, insulin sensitivity, nutrition, pregnancy

1 | INTRODUCTION

Bile acids (BA) are mainly known as facilitators for lipid absorption and for their role in cholesterol excretion.[1] However, as endocrine signaling molecules, BA also act in the regulation of various metabolic processes such as cholesterol regulation, triglyceride, glucose, and energy homeostasis.[2,3] Two main receptors are involved in the previously mentioned metabolic pathways. The first one is the nuclear receptor, farnesoid X receptor (FXR), a hepatic and intestinal receptor activated by BA whose main function is the retro-inhibition of BA synthesis in the liver.[4] Interestingly, FXR plays a key role in glucose homeostasis, as FXR agonists contribute to an increase in glucose output from hepatocytes.[5]

The second receptor is the membrane-bound G-protein-coupled bile acid receptor (TGR5), responsible for glucagon-like peptide-1 (GLP-1) secretion.[6] GLP-1 is responsible for the increase of glucose-stimulated insulin secretion. TGR5 is also expressed in brown adipose tissue and muscle and liver cells, therefore making it a modulator of energy expenditure.[6]

Among the previously enounced pathways, glucose homeostasis mechanisms are of great interest to the understanding of BA metabolic influence. It has been shown that FXR and TGR5 activation can respectively alter blood glucose levels and endogenous insulin secretion.[7,8] Studies on mice have shown that hepatic FXR-BA activation begets a decrease in hepatic gluconeogenesis paired with a rise in hepatic glycogen synthesis.[9,10] Moreover, TGR5-BA activation has been associated with GLP-1 secretion and therefore improved glucose homeostasis.[11,12]

The BA pool itself undergoes metabolic alterations, notably during pregnancy. Studies in pregnant mice and women have shown a general and steady rise in BA concentrations throughout gestation.[13-15] Moreover, in some women, raised BA levels may become pathological, resulting in intrahepatic cholestasis of pregnancy (ICP) in the second and third trimester.[16] Concomitantly, gestation modifies glucose homeostasis from the first trimester to the end of pregnancy. These early glucose homeostasis adaptations in the first trimester are predictive of increased insulin resistance in the second and third trimesters, which can become pathological in some cases and lead to gestational diabetes mellitus.[8,17] However, few studies have focused on the relation between both factors during pregnancy.

To this end, a better understanding of BA changes in pregnancy could help understand their impact on glucose regulation. Therefore, this study aims to, first, characterize the changes in BA metabolism across trimesters and, second, evaluate associations between the BA profile and glucose homeostasis indexes in the first and the second trimesters of pregnancy.

2 | MATERIAL AND METHODS

This is a secondary analysis of data from the ANGE (*Apports Nutritionnels durant la GrossessE*) cohort, a study that aimed to assess nutritional intakes during pregnancy and in which a total of 86 healthy pregnant women were recruited at the *CHU de Québec - Université Laval* (Québec City, Canada) research center and followed prospectively throughout pregnancy. The study protocol was previously described.[18] Briefly, women were included if they were at least 18 years of age and had a gestational age inferior or equal to 11 weeks. Women previously diagnosed with an inflammatory or autoimmune disorder, type 1 or 2 diabetes or a renal disease were excluded.

2.1 | Measurement of serum BA

Fasting serum samples were collected in the first (week 12.5 ± 0.7), second (week 22.5 ± 0.8) and third trimesters (week 33.0 ± 0.5) of pregnancy. Serum BA profiling was performed using liquid chromatography with tandem mass spectrometry (LC-MS/MS) as previously described, using a Nexera ultra-high pressure liquid chromatography (UHPLC) instrument (Shimadzu Scientific Instruments, Columbia, Maryland) and the previously reported chromatographic method.[19,20] This method allowed the quantitative measurement of 30 different bile acid molecules including the unconjugated (CDCA, CA, LCA, DCA, UDCA, HDCA, 3dhLCA, and HCA), taurine-conjugated (TCDCA, TCA, TLCA, TDCA, and TUDCA), glycine-conjugated (GCDCA, GCA, GDCA, GLCA, and GUDCA) as well as glucuronide-conjugated forms (CDCA-3 and -24G, CA-24G, LCA-3, and -24G, DCA-3, and -24G, HDCA- and HCA-6 and -24G; Table S1).

2.2 | Measurement of glucose homeostasis indexes

A 75 g oral glucose tolerance test was performed in the first and second trimesters. Glucose, C-peptide, and insulin levels were monitored throughout the test by blood sampling at several timepoints: −15, 0, 15, 30, 60, 90, and 120 minutes. Plasma glucose was measured enzymatically by the hexokinase method (Siemens, Dimension Vista 1500, CV 1.8%) whereas insulin and C-peptide were measured with an electrochemiluminescence immunoassay (Siemens, Advia Centaur XPT, CV 3.5, and 5%, respectively). The obtained data was used to calculate the following indexes: homeostasis model assessment of insulin resistance (HOMA-IR: Fasting insulin[μU/ml]x fasting glucose [mmol/L])/22.5), Matsuda index (10 000/[fasting glucose × fasting insulin × (mean glucose × mean insulin)]$^{1/2}$), insulinogenic index ([C-peptide 30 minutes—fasting C-peptide]/[glucose 30 minutes—fasting glucose]). Incremental area under the curve for C-peptide (iAUC C-Peptide) was calculated using the trapezoid method from 0 to 120 minutes. Disposition index was calculated using Matsuda index × iAUC C-Peptide.

2.3 | Other variables

Prepregnancy body mass index (BMI; weight (kg)/height (m)^2) was calculated using self-reported prepregnancy body weight and on-site measured height. Participants completed a socio-economic Web questionnaire during their pregnancy.

2.4 | Bile acids analysis

As previously reported,[21] total BA concentrations correspond to the sum of all BA measured. Total sums of glyco-, tauro-, glucuronide-conjugates were calculated by the summation of concentrations of conjugated BA: CDCA, CA, DCA, UDCA, and LCA. The sum of unconjugated BA included HDCA, 3dhLCA, and HCA concentrations. The total of primary, secondary, and hydroxylated BA species was determined by adding all unconjugated and/or conjugated species of CDCA + CA, LCA + DCA, or HDCA + HCA, respectively. Finally, the relative proportion of a specific bile acid corresponds to its concentrations divided by the total concentration of BA. For this study, only the following subcategories of BA (total BA, total unconjugated, total glyco-and tauro-conjugated BA, total primary and total secondary BA, total CA and total CDCA) were analyzed.

2.5 | Statistical analysis

Descriptive analyses were conducted to characterize the study population. For all continuous variables, repeated measures multivariate analyses of variance (MANOVAs) with Tukey's honest significant difference (HSD) posthoc tests for multiple comparisons were performed to assess differences in total BA, total unconjugated and total glyco- and tauro-conjugated BA, primary and secondary BA and CA and CDCA dosing across trimesters. Pearson correlation analysis were conducted to assess the associations between each category of BA and each glucose homeostasis marker for normal or normalized distributions using log or box-cox transformations. Spearman's nonparametric correlation analyses were conducted on nonnormal distributions. Differences and correlations were considered statistically significant at $P < .05$. All statistical analyses were performed using JMP software (version 14, SAS Institute Inc, Cary, North Carolina).

2.6 | ETHICS STATEMENT

The ANGE project was approved by the *CHU de Québec - Université Laval* Research Center's Ethical Committee on March 7, 2016 (reference number: 2016-2866) and, for the present analyses, data were available for 78 of the 86 initially recruited women. All participants gave their informed written consent at their first visit to the research center.

3 | RESULTS

Participants' characteristics are presented in Table 1. Our final analyses include 78 women who completed all trimester visits, aged 32.1 ± 3.7 years old and with a mean prepregnancy BMI of 25.7 ± 5.8 kg/m^2. Most participants were Caucasian, multiparous, and had a high socioeconomic status. A small percentage of participants had gestational diabetes mellitus (12.8%). No participant had ICP (data not shown).

Figure 1 illustrates the concentration of several BA throughout trimesters. Significant rises in concentrations from the first trimester to the end of pregnancy were observed for total BA, total glycine- and taurine-conjugated BA, total secondary BA, and total cholic acids (i.e., CA + TCA + GCA). A significant and linear rise of the ratio total CA: total CDCA ratio was also observed throughout trimesters. Figure 2 shows changes between trimesters in relative abundance of conjugated and unconjugated acids within the total BA pool. Significant decreases in relative abundance of total unconjugated BA were observed in the third trimester (27.45% in the third trimester compared to 35.45% in the first trimester, $P < .0001$), hence lowering the total unconjugated: total glycol, tauro, and glucurono-conjugated BA ratio.

Correlations between specific BA categories and glucose homeostasis markers for the first and second trimesters are shown in Table 2. Significant positive correlations were observed between HOMA-IR and total BA in the first and second trimesters ($r = .23$, $P = .04$ for the first trimester and $r = .40$ $P = .0003$ for the second trimester). Significant inverse correlations were observed with the Matsuda index ($r = -.23$, $P = .046$ for the first trimester and $r = -.38$,

TABLE 1 Participants' characteristics (n = 78)

Variables	Mean ± SD or N (%)
Age (years)	32.1 ± 3.7
Prepregnancy BMI (kg/m^2)	25.7 ± 5.8
Primiparous	28 (35.9)
Ethnicity-Caucasian	76 (97.4)
Education	
High school	3 (3.8)
College	13 (16.7)
University	62 (79.5)
Household annual income (Canadian dollars)	
<$40 000	5 (6.4)
$40 000 to $59 999	10 (12.8)
$60 000 to $79 999	13 (16.7)
$80 000 to $99 999	16 (20.5)
≥$100 000	33 (42.3)
Income missing	1 (1.3)
Gestational diabetes mellitus	10 (12.8)
HOMA-IR index	
1st trimester	2.14 ± 1.08
2nd trimester[a]	2.49 ± 1.40
Matsuda index	
1st trimester[a]	5.56 ± 2.19
2nd trimester	4.75 ± 2.76
Insulinogenic index	
1st trimester[c]	901.8 ± 1833.4
2nd trimester[b]	855.2 ± 948.9
iAUC C-Peptide (x10^5)	
1st trimester	2.02 ± 0.52
2nd trimester[b]	1.47 ± 0.45
Disposition index (x10^5)	
1st trimester	10.41 ± 3.33
2nd trimester[a]	5.97 ± 2.18

Abbreviations: BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin; iAUC C-Peptide, incremental area under the curve for C-Peptides.
[a]n = 77.
[b]n = 76.
[c]n = 75.

P = .0006 for the second trimester). In the first and second trimesters, higher levels of total primary BA were associated with lower values for insulin sensitivity indexes (r = –.23, P = 0.045 for the first trimester and r = –.35, P = .002 for the second trimester).Furthermore, insulin resistance indexes were positively correlated with all BA categories and sensitivity indexes were inversely correlated with almost all BA categories, in the second trimester. In sum, higher concentrations of BA correlated with lower insulin sensitivity. As for insulin secretion indexes, few associations of significant interest were established, as most of them were isolated and weakly correlated. However, iAUC C-Peptide correlated positively and significantly with total BA in the second trimester (r = .25, P = .03) and with other subcategories of BA (see Table 2).

Table 3 shows correlations changes in BA concentrations and changes in insulin resistance indexes from the first to the second trimester. Variations in HOMA-IR were positively and significantly correlated with variations in all analyzed BA categories. No other glucose homeostasis marker's variation was correlated with variations of specific BA categories, except total CA changes that were significantly associated with iAUC C-Peptide changes (r = .23, P = .04).

No association was observed between any of the BA concentrations and the prevalence of gestational diabetes mellitus. Furthermore, no significant associations were detected between BA changes and prepregnancy BMI, total or trimester-specific weight gain, age, ethnicity, or primiparity.

4 | DISCUSSION

Our results provide novel insights into the understanding of BA metabolism during pregnancy. First, we found that concentrations of total and subcategories BA increased consistently and significantly across pregnancy, except for total unconjugated BA, primary BA, and CDCA. Thus, we demonstrated that there are physiological changes in circulating BA during pregnancy. Second, multiple significant although weak correlations were found between trimester-specific BA concentrations and same-trimester insulin sensitivity indexes, particularly in the second trimester. The strongest correlations observed were those between all BA categories and HOMA-IR, particularly between total BA and HOMA-IR. Third, we found that changes in total BA concentrations correlated positively with changes in HOMA-IR between the first and second trimesters. We can thus hypothesize that serum BA concentrations and hepatic insulin resistance have a similar evolution in early pregnancy up to the second trimester.

Briefly, our results align with the current knowledge of BA changes during pregnancy regarding total BA, total CA: total CDCA ratio and total conjugated: total unconjugated BA ratio.[8,15] As such, a similar study performed in a cohort of pregnant Chinese women found significant increases of serum BA concentrations and changes similar to those observed in our study for total conjugated: total unconjugated BA ratios, both between the second and third trimesters. However, only 14 BA were analyzed in solely the second and third trimesters and different cohorts were used for each trimester, as opposed to the present study where the same women were followed prospectively.[15] More precisely, our results demonstrated a decrease in total unconjugated: total conjugated BA ratio, especially from the second to the third trimester. Interestingly, conjugated BA are less hydrophobic than unconjugated BA and therefore less toxic.[22] Although the physiological explanations behind this change in the BA pool remain to be elucidated, the accentuated rise in hydrophilic and less toxic conjugated acids could be a defense mechanism activated in response to the rise in total BA. The rise in glyco- and tauro-conjugated BA occurs only in noncholestatic pregnant women

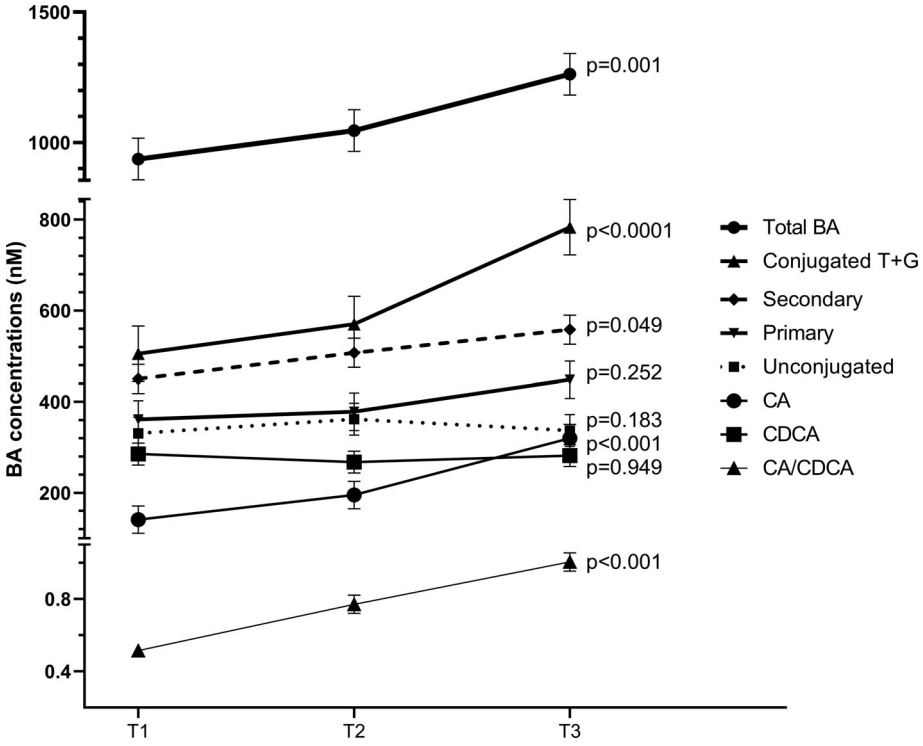

FIGURE 1 Changes in BA concentrations throughout pregnancy. T1; 1st trimester, T2; 2nd trimester, T3; 3rd trimester, BA; total BA, Conjugated T+G; total glycine and taurine conjugated BA, Secondary; secondary BA, Primary; primary BA, Unconjugated; total unconjugated BA, CA; total cholic acid, CDCA; total chenodeoxycholic acid, CA/CDCA; total cholic/total chenodeoxycholic acid ratio. Values are presented as means ± SE (n = 78)

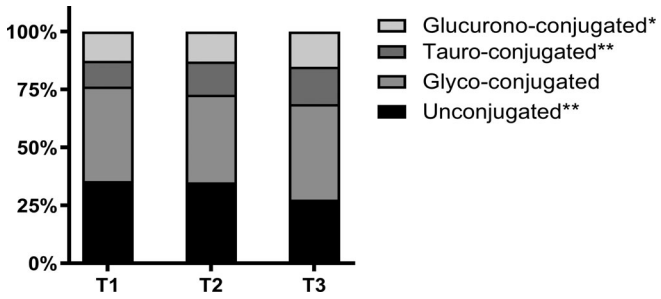

FIGURE 2 Relative abundance of conjugated forms of BA within the total pool. T1; 1st trimester, T2; 2nd trimester, T3; 3rd trimester. *, Significant 1st to 3rd trimester variations with $P = .01$. **, Significant 1st to 3rd trimester variations with $P < .0001$

according to a specific cross-sectional study, in which 30 women with a healthy pregnancy were compared with 41 women with ICP and 10 nonpregnant women. Fasting serum samples were collected in the third trimester for both groups. Women with ICP presented higher levels of free BA than healthy pregnant women, whom instead had higher levels of glyco- and tauro-conjugated BA. Furthermore, healthy pregnant women had higher levels of serum conjugated BA compared to nonpregnant women.[14] The results are therefore similar to those found in our study.

We also observed a significant rise in CA concentrations throughout trimesters. That rise could be caused by an inhibition of FXR, which has been associated with an increase in the expression of human sterol 12α-hydroxylase gene (Cyp8b1), a gene involved in CA synthesis under the regulation of FXR.[23] Furthermore, CDCA concentrations in our study did not change throughout pregnancy, meaning the CA:CDCA ratio increased, attenuating the BA pool toxicity.[22] Interestingly, elevation in CA:CDCA ratio paired with raised total BA concentrations are typically characteristic of ICP incidence.[24,25] However, none of our participants were diagnosed with ICP, despite having increased CA:CDCA ratio and increased total BA. Hence, the rise observed in CA:CDCA ratio in the present study may be physiological rather than pathological in cases of normal pregnancy. Finally, our results show a greater increase in all BA categories concentrations in the last two trimesters. This onset might be due to the foetus's capacity to produce but not metabolize BA properly. Recent research has shown that BA metabolites are released from the placenta to the bloodstream during the second and third trimesters in order to be metabolized by the maternal liver, increasing significantly serum BA concentrations in the mother.[26,27]

The multiple significant correlations found between BA subcategories and hepatic insulin sensitivity markers provide interesting insights on the impacts of elevated BA production. Theoretically, activated FXR inhibits the pathway leading to glucose release from the liver into the bloodstream.[5,28] Similarly, endogenous insulin inhibits hepatic gluconeogenesis. Hence, hepatic insulin sensitivity indexes indicate the presence or absence of gluconeogenesis. Our results show positive associations between total BA concentrations and HOMA-IR score, meaning that raised BA concentrations may be associated with raised hepatic insulin resistance through an increase in gluconeogenesis. Furthermore, BA concentrations correlate negatively with the Matsuda index, a "whole body" insulin sensitivity marker. It has been shown that FXR activation is involved in the increase of peripheral insulin sensitivity in mice models.[28] Similarly, literature shows that TGR5 ligands contribute to higher energy expenditure and therefore to glucose storage in peripheral tissues, hence increasing

TABLE 2 Pairwise correlations between BA categories and glucose homeostasis indexes in the first and the second trimester

	Insulin sensitivity indexes				Insulin secretion indexes					
	HOMA-IR		Matsuda index		Insulinogenic index		iAUCC-Peptide		Disposition index	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
1st trimester										
Total BA	.23	**.04**	−.23	**.046**	.17	.14	.21	.07	−.11	.33
Unconjugated	.35	**.002**	−.29	**.01**	.19	.11	.16	.15	−.25	**.03**
Conjugated T + G	.08	.47	−.14	.22	.10	.38	.22	.06	.02	.85
Primary	.23	**.04**	−.23	**.045**	.14	.25	.20	.08	−.14	.23
Secondary	.18	.11	−.19	.11	.14	.23	.18	.11	−.07	.55
CA	.20	.08	−.19	.09	−.02	.89	.17	.13	−.12	.30
CDCA	.20	.08	−.21	.07	−.16	.17	.20	.08	−.10	.37
2nd trimester										
BA	.40	**.0003**	−.38	**.0006**	.20	.08	.25	**.03**	−.32	.05
Unconjugated	.32	**.004**	−.22	.05	.12	.32	.06	.62	−.30	.08
Conjugated T + G	.38	**.0007**	−.40	**.0003**	.22	.06	.30	**.008**	−.25	**.03**
Primary	.37	**.0008**	−.35	**.002**	.20	.08	.23	**.04**	−.29	**.01**
Secondary	.34	**.002**	−.32	**.005**	.16	.16	.22	.05	−.27	**.02**
CA	.29	**.009**	−.24	**.03**	.12	.32	.21	.06	−.18	.12
CDCA	.38	**.0006**	−.40	**.0003**	.24	**.04**	.23	**.046**	−.32	.06

Note: Total BA; total BA, Conjugated T + G; total glycine and taurine conjugated BA, Secondary; secondary BA, Primary; primary BA, Unconjugated; total unconjugated BA, CA; total cholic acid, CDCA; total chenodeoxycholic acid, HOMA-IR; homeostasis model assessment of insulin resistance index, iAUC C-Peptide; incremental area under the curve for C-peptides. Bold values indicates significant results ($P < 0.05$).

TABLE 3 Pairwise correlations between BA changes and glucose homeostasis indexes changes from the 1st to the 2nd trimester

	Insulin sensitivity indexes				Insulin secretion indexes					
	HOMA-IR		Matsuda index		Insulinogenic index		iAUCC-Peptide		Disposition index	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
BA	.35	**.002**	−.23	.05	.13	.27	.12	.3	−.04	.75
Unconjugated	.28	**.01**	−.22	.06	.13	.23	.09	.42	−.12	.32
Conjugated T + G	.30	**.008**	−.18	.12	.12	.33	.11	.37	−.02	.89
Primary	.31	**.007**	−.22	.06	.09	.46	.15	.19	−.08	.51
Secondary	.35	**.002**	−.19	.1	.12	.31	.10	.33	−.01	.97
CA	.24	**.04**	−.19	.11	.04	.74	.23	**.04**	−.02	.89
CDCA	.30	**.009**	−.19	.09	.13	.26	.10	.41	−.04	.76

Note: Total BA; total BA, Conjugated T + G; total glycine and taurine conjugated BA, Secondary; secondary BA, Primary; primary BA, Unconjugated; total unconjugated BA, CA; total cholic acid, CDCA; total chenodeoxycholic acid, HOMA-IR; homeostasis model assessment of insulin resistance index, iAUC C-Peptide; incremental area under the curve for C-peptides.

peripheral insulin sensitivity.[29] As BA serum concentrations and therefore possibly BA synthesis are increased during pregnancy, it might be possible that pregnancy-related physiological changes cause to FXR inhibition, leading to increased hepatic glucose output despite normal insulin secretion. We could similarly hypothesize that physiological pregnancy leads to TGR5 inhibition, as we see a decrease in peripheral insulin sensitivity despite an increase in TGR5 agonists (secondary BA). Hence, this inhibition of FXR and TGR5 could be a protective mechanism developed to voluntarily increase insulin resistance, which is typical in late pregnancy and results in increased circulating glucose and free fatty acids available for fetal growth.[30]

Few significant associations were observed between BA categories and insulin secretion markers. These results go against an association between insulin secretion and BA concentrations in physiologic pregnancies. Other studies have shown that the TGR5-BA activation induces intestinal GLP-1 secretion, which in turn induces insulin secretion in the pancreatic islets β-cells.[6] Coincidently, our data shows

a significant rise in secondary BA throughout pregnancy, the molecules with the most potency for TGR5. This data could however be interpreted, as previously enounced, as a sign of potential TGR5 inhibition by physiological changes of pregnancy. Further research is therefore needed to elucidate mechanisms that could potentially link physiological pregnancy and BA-activated receptors.

Our research shows the potential role of BA regulation in glucose homeostasis during pregnancy, as it is one of the few studies to monitor BA changes in association with glucose homeostasis alterations for the first two trimesters of pregnancy with a sufficient sample size. Dosing of BA subcategories and use of multiple insulin sensitivity and insulin secretion indexes allowed us to precisely characterize the nature of the associations between BA and glucose regulation during pregnancy. However, we could not compare BA profile with glucose regulation in late pregnancy as we did not perform an oral glucose tolerance test during the third trimester on-site visit. This is one of the limitations of our study, as glucose homeostasis alterations occur generally from the second trimester to the end of gestation. The lack of representativeness of our population sample is also a limitation to our study, since most of recruited women were Caucasian and had a high socioeconomic status. Finally, the small proportion of women who developed gestational diabetes mellitus did not allow us to observe proper differences in the BA profile compared to normal pregnant women, hence the lack of causality between BA changes and pathological repercussions.

5 | CONCLUSION

This study is the first one to extensively monitor detailed BA profile in a longitudinal context during pregnancy and it provides a reference point for gestational changes in BA profile in healthy women. The observed modifications in the BA pool may appear as early as in the first trimester visit, meaning they could be monitored in order to foresee BA changes in the latter stages of pregnancy. Furthermore, the significant correlations found between variations in BA concentrations' and insulin resistance elevations in the first two trimesters led us to believe that changes in the BA pool and glucose homeostasis are related. In light of these findings, further research is needed to fully understand the mechanisms underlying these associations and whether BA could be used as a therapeutic target in adverse pregnancy outcomes such as gestational diabetes mellitus and ICP.

ACKNOWLEDGMENTS

The authors would like to acknowledge the contribution of trained dietitian and research assistant Anne-Sophie Plante for the revision of this manuscript, as well as the research team behind the data collection used for this secondary analysis, including Claudia Savard and Marjorie Labrecque.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

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Writing - Review and Editing: S. John Weisnagel, Claudia Gagnon, Olivier Barbier, Anne-Marie Carreau, Anne-Sophie Morisset
Investigation: Jocelyn Trottier, Olivier Barbier
Methodology: Jocelyn Trottier
Validation: Anne-Marie Carreau, Olivier Barbier, Anne-Sophie Morisset
Supervision: Anne-Sophie Morisset

All authors have read and approved the final version of the manuscript.

Anne-Sophie Morisset had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY STATEMENT

Anne-Sophie Morisset affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Gagnon M, Trottier J, Weisnagel SJ, et al. Bile acids during pregnancy: Trimester variations and associations with glucose homeostasis. *Health Sci Rep*. 2021;4:e243. https://doi.org/10.1002/hsr2.243