Intrahepatic cholestasis of pregnancy (ICP) is a rare liver disorder which usually manifests in the third trimester and associated with increased perinatal morbidity and mortality. The hallmark laboratory abnormality in ICP is elevated fasting serum bile acids; however, there are limited data on whether a nonfasting state affects a pregnant woman’s total bile acids. This study assesses fasting and nonfasting bile acid levels in 10 healthy pregnant women after a standardized glucose load to provide insight into the effects of a glucose load on bile acid profiles.

**Study Design**

Pilot prospective cohort analysis of serum bile acids in pregnant women. A total of 10 healthy pregnant women from 28 to 32 weeks’ gestation were recruited for the study before undergoing a glucose tolerance test. Total serum bile acids were collected for each subject in the overnight fasting state, and 1 and 3 hours after the 100-g glucose load.

**Results**

There was a statistically significant difference between fasting versus 3-hour values. There was no statistically significant difference between fasting versus 1-hour and 1-hour versus 3-hour values.

**Conclusion**

There is a difference between fasting and nonfasting total serum bile acids after a 100-g glucose load in healthy pregnant women.
may have a strong influence on the disease process.\textsuperscript{1,2,16,17} There may also be subclinical physiologic cholestasis during pregnancy, which is exacerbated in individuals with some genetic or environmental predisposition.\textsuperscript{18}

Elevation of total bile acids (TBA) is the most frequent laboratory abnormality associated with ICP and is the most sensitive marker for ICP.\textsuperscript{1,14,15,18,19} Bile acids are the end products of cholesterol catabolism with their most recognized functions being to aid in the digestion and absorption of fatty acids in the intestine. Bile acids are actively secreted by the liver into bile and stored in the gall bladder, which releases them into the lumen of the intestine after ingestion of the meal. The efficiency of the hepatic clearance of bile acids from portal blood maintains serum concentrations at low levels in normal persons. Therefore, an elevated fasting level, because of the impaired hepatic clearance, is a sensitive indicator of liver disease.\textsuperscript{7,17,20,21} Following meals, serum bile acid levels have been shown to increase only slightly in normal persons, but markedly in patients with various liver diseases, including cirrhosis, hepatitis, cholestasis, portal-vein thrombosis, Budd-Chiari syndrome, cholangitis, Wilson disease, and hemochromatosis.\textsuperscript{17,20,22}

Despite the potential impact of bile acids on fetal morbidity and mortality, there is a paucity of information regarding the temporal changes of serum bile acids during pregnancy. This is confounded by not only the wide range of values for the diagnosis of ICP in the current literature, but also the fact that the current reference ranges are generally for fasting values of TBA and generally exclude values from pregnant subjects.\textsuperscript{5,11,16,23–28} Confusing the situation further, while elevated fasting serum bile acids is the most sensitive marker for the diagnosis of ICP, many obstetricians order serum bile acids when the clinical suspicion for ICP arises and the patient is very unlikely to be fasting.\textsuperscript{12,13} To understand ICP, it is important to understand the relationship between fasting TBA and nonfasting TBA in pregnant women. We sought to determine the impact of a glucose load on the TBA levels in healthy pregnant women and hypothesized that the nonfasting state would transiently elevate TBA, which may complicate the diagnosis of ICP.

**Methods**

This prospective pilot study evaluated the fasting and nonfasting TBA levels of healthy pregnant women at Hennepin County Medical Center, Minneapolis, MN from September 2012 to January 2013. This study was approved by the Institutional Review Board (HSR number 12–3436). Women were recruited for the study if they were undergoing a 3-hour glucose tolerance test.

Overall, 10 healthy pregnant women older than 18 years with a singleton pregnancy who were undergoing a 3-hour glucose tolerance test and confirmed to be fasting were recruited and enrolled in the study. Pregnant women with a history of liver disease or gall bladder disease, including hepatitis, alcohol abuse, and history of gastric bypass surgery or current unexplained pruritus were excluded. All women were given a copy of the study consent form and written informed consent was obtained. Those who met study inclusion criteria also completed a short survey which collected information on demographics and medical, surgical, and family history.

Serum TBA levels were collected in the overnight fasting state and then 1 and 3 hours after a 100 g glucose load. The samples were then processed and stored at –20°C until all 10 recruited patients had delivered. The frozen serum samples were analyzed via enzymatic colorimetric assay (ARUP laboratories in Salt Lake City, UT) for the determination of TBA. The TBA reference interval of 0 to 10 µmol/L was based on fasting specimens.

Demographic and medical history data were summarized and reported as frequencies and percentages or mean ± standard deviation (SD) as appropriate unless otherwise indicated. Serum TBA levels were compared over time using a repeated measures analysis of variance (ANOVA) model assuming a compound symmetry covariance structure and pairwise comparisons were conducted. As this was a pilot study, p values were not adjusted for multiple comparisons. All analyses were performed using SAS (Version 9.3, Cary, NC) and p values < 0.05 were considered statistically significant.

**Results**

Characteristics of the 10 study participants are described in Table 1. Of the participants, five (50%) were multiparous and five (50%) were primaparous. The study population was predominately Caucasian (40%) and the mean gestational age at enrollment was 29 weeks ± 6 days (range, 28 weeks ± 1 day to 31 weeks ± 1 day). The mean gestational age at delivery of the cohort was 39 weeks ± 4 days (range, 37 weeks ± 0 days to 41 weeks ± 3 days).

Mean serum TBA levels increased over time from 3.4 ± 2.1 µmol/L at fasting, 4.2 ± 2.5 µmol/L at 1 hour post glucose load, and 5.6 ± 3.7 µmol/L at 3 hours post glucose load (Fig. 1). There was a statistically significant change in serum TBA levels over time (F2,18 = 3.42, p = 0.05). Specifically, while the differences in serum TBA levels between the fasting and 1 hour (p = 0.36) and 1 and 3 hours (p = 0.12) were not statistically significant, the mean serum TBA levels at 3 hours were statistically significantly higher than the fasting serum TBA levels (p = 0.02).

**Discussion**

Our pilot study investigated the effects of a glucose load on serum TBA levels in healthy pregnant women. There is currently a lack of information regarding the effects of the nonfasting state on serum TBA in pregnant subjects. Our study is one of the first to compare fasting and nonfasting levels of TBA in a healthy pregnant population in the United States. Our results highlight the small, but statistically and likely clinically significant difference in serum TBA between fasting and 3-hour post glucose load samples in a small population of healthy pregnant women. Barnes et al reported that in the healthy nonpregnant population, TBA levels return to fasting levels shortly after a meal,\textsuperscript{20} however, in our study, the mean TBA
levels remained significantly higher than fasting values at 3 hours. This result is consistent with observations that normal pregnancy is a cholestatic state, as it has been reported that up to 40% of asymptomatic women may have a postprandial TBA value > 10 µmol/L. Should postprandial serum TBA levels be used to diagnose ICP? Our results indicate that even in the healthy pregnant population, the postprandial state affects serum TBA levels for hours. Fasting TBA levels may be the most sensitive indicator of severe liver disease for pregnant patients as postprandial levels may be elevated at baseline. However, there may be a role for the interpretation of nonfasting TBA, as elevated TBA in the nonfasting state from delayed clearance may be an early indicator of mild disease.

Overall, this pilot study, while small, suggests that in our population the standard fasting reference ranges for serum TBAs are appropriate. This is consistent with previous studies to determine the reference range for total serum bile acids in the pregnant population. This study also provides important initial data on how a glucose load affects the range of bile acids in the healthy pregnant population and it appears that fasting serum TBA levels remain the most appropriate form of evaluation. These data will assist in resolving the current diagnostic dilemma with ICP, in particular, when should the obstetrician obtain the specimen for serum TBAs as well as how to determine the appropriate normal range in the pregnant population. Further studies using a lipid containing test meal would provide more physiologic data and a larger study is needed to further support these findings.

Note
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References
1. Pathak B, Sheibani L, Lee RH. Cholestasis of pregnancy. Obstet Gynecol Clin North Am 2010;37(2):269–282
2. Bull LN, Vargas J. Serum bile acids in intrahepatic cholestasis of pregnancy: not just a diagnostic test. Hepatology 2014;59(4):1220–1222
3. Floreani A, Caroli D, Lazzari R, et al. Intrahepatic cholestasis of pregnancy: new insights into its pathogenesis. J Matern Fetal Neonatal Med 2013;26(14):1410–1415
4. Yoong W, Memtsa M, Pun S, West P, Loo C, Okolo S. Pregnancy outcomes of women with pruritus, normal bile salts and liver enzymes: a case control study. Acta Obstet Gynecol Scand 2008;87(4):419–422
5. Webb GJ, Elsharkawy AM, Hirschfield GM. The etiology of intrahepatic cholestasis of pregnancy: towards solving a monkey puzzle. Am J Gastroenterol 2014;109(1):85–88
6. Chen J, Deng W, Wang J, Shao Y, Ou M, Ding M. Primary bile acids as potential biomarkers for the clinical grading of intrahepatic cholestasis of pregnancy. Int J Gynaecol Obstet 2013;122(1):5–8
7. Mullally BA, Hansen WF. Intrahepatic cholestasis of pregnancy: review of the literature. Obstet Gynecol Surv 2002;57(1):47–52
Tribe RM, Dann AT, Kenyon AP, Seed P, Shennan AH, Mallet A. Longitudinal profiles of 15 serum bile acids in patients with intrahepatic cholestasis of pregnancy. Am J Gastroenterol 2010; 105(3):585–595

Kondrackiene J, Beuers U, Zalinkevicius R, Tauschel HD, Gintautas V, Kucinskas L. Predictors of premature delivery in patients with intrahepatic cholestasis of pregnancy. World J Gastroenterol 2007; 13(46):6226–6230

Geenes V, Chappell LC, Seed PT, Steer PJ, Knight M, Williamson C. Association of severe intrahepatic cholestasis of pregnancy with adverse pregnancy outcomes: a prospective population-based case-control study. Hepatology 2014;59(4):1482–1491

Arthur C, Mahomed K. Intrahepatic cholestasis of pregnancy: diagnosis and management; a survey of Royal Australian and New Zealand College of Obstetrics and Gynaecology fellows. Aust N Z J Obstet Gynaecol 2014;54(3):263–267

Walker IA, Nelson-Piercy C, Williamson C. Role of bile acid measurement in pregnancy. Ann Clin Biochem 2002;39(Pt 2):105–113

Walker I, Chappell LC, Williamson C. Abnormal liver function tests in pregnancy: diseases unique to pregnancy. World J Gastroenterol 2013;19(43):7639–7646

Lee RH, Ouzounian JC, Goodwin TM, et al. Bile acid concentration reference ranges in a pregnant Latina population. Am J Perinatol 2013;30(5):389–393

Lee RH, Goodwin TM, Greenspoon J, Incerpi M. The prevalence of intrahepatic cholestasis of pregnancy in a primarily Latina Los Angeles population. J Perinatol 2006;26(9):527–532

Frisé CJ, Williamson C. Gastrointestinal and liver disease in pregnancy. Clin Med 2013;13(3):269–274

Bacq Y, Zarka O, Bréchet JF, et al. Liver function tests in normal pregnancy: a prospective study of 103 pregnant women and 103 matched controls. Hepatology 1996;23(5):1030–1034

Lunzer M, Barnes P, Byth K, O’Halloran M. Serum bile acid concentrations during pregnancy and their relationship to obstetric cholestasis. Gastroenterology 1986;91(4):825–829

Barnes S, Gallo GA, Trash DB, Morris JS. Diagnostic value of serum bile acid estimations in liver disease. J Clin Pathol 1975;28(6):506–509

Carter J. Serum bile acids in normal pregnancy. Br J Obstet Gynaecol 1991;98(6):540–543

Glantz A, Marshell HU, Mattsson LA. Intrahepatic cholestasis of pregnancy: Relationships between bile acid levels and fetal complication rates. Hepatology 2004;40(2):467–474

Wong LFA, Shallow H, O’Connell MP. Comparative study on the outcome of obstetric cholestasis. J Matern Fetal Neonatal Med 2008;21(5):327–330

Ahmed KT, Almashhrawi AA, Rahman RN, Hammoud GM, Ibda JA. Liver diseases in pregnancy: diseases unique to pregnancy. World J Gastroenterol 2013;19(43):7639–7646

Brites D, Rodrigues CM, van-Zeller H, Brito A, Silva R. Relevance of serum bile acid profile in the diagnosis of intrahepatic cholestasis of pregnancy in an high incidence area: Portugal. Eur J Obstet Gynecol Reprod Biol 1998;80(1):31–38

Palma J, Reyes H, Ribaltà J, et al. Ursodeoxycholic acid in the treatment of cholestasis of pregnancy: a randomized, double-blind study controlled with placebo. J Hepatol 1997;27(6):1022–1028

Abbassi-Ghanavati M, Greer LG, Cunningham FG. Pregnancy and laboratory studies: a reference table for clinicians. Obstet Gynecol 2009;114(6):1326–1331

Fulton IC, Douglas JG, Hutchon DJR, Beckett GJ. Is normal pregnancy cholestatic? Clin Chim Acta 1983;130(2):171–176

Castaño G, Lucangioli S, Sookoian S, et al. Bile acid profiles by capillary electrophoresis in intrahepatic cholestasis of pregnancy. Clin Sci (Lond) 2006;110(4):459–465

Heikkinen J. Effect of a standard test meal on serum bile acid levels in healthy nonpregnant and pregnant women and in patients with intrahepatic cholestasis of pregnancy. Ann Clin Res 1983;15(5-6):183–188

Laatikainen T, Ikonen E. Serum bile acids in cholestasis of pregnancy. Obstet Gynecol 1977;50(3):313–318