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Women's periconceptional diet and risk of biliary atresia in offspring

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Abstract

INTRODUCTION—We examined the association of biliary atresia with maternal dietary intake, using National Birth Defects Prevention Study (NBDPS) data from 152 cases and 11,112 non-malformed controls born 1997–2011.

METHODS—NBDPS is a multi-site, population-based case-control study. Exposure data were from maternal telephone interviews, which included a food frequency questionnaire. Odds ratios (OR) and 95% confidence intervals (CI) were generated from logistic regression models that included nutritional factors as continuous variables and were adjusted for energy intake only or energy intake plus covariates (maternal race-ethnicity, education, age, pre-pregnancy body mass index, vitamin/mineral supplement intake, conception during summer). Models included a quadratic term for the nutrient if $p<0.10$. ORs reflect odds of having biliary atresia for nutrient values at the 75th compared to 25th percentile values of each nutrient, based on distributions among controls.

RESULTS—ORs for which the 95% CI excluded 1.00 were energy-adjusted ORs for calcium (0.63), protein (0.65), riboflavin (0.71), and diet quality index (0.69), and fully adjusted ORs for calcium (0.68) and vitamin E (0.72). ORs that were fully adjusted for covariates tended to be closer to 1.0 than ORs adjusted only for energy intake. ORs for the other studied nutrients had 95% CIs that included 1.00.

CONCLUSIONS—NBDPS is the first study to include detailed information on maternal dietary intake and risk of biliary atresia. Our results suggest reduced risks associated with some nutrients,

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

which may provide etiologic clues but should be interpreted with caution given the small number of cases and novelty of the investigation.

Keywords

nutrition; biliary atresia; birth defects; epidemiology; vitamins; maternal

INTRODUCTION

Biliary atresia refers to a neonatal condition in which the passage of bile from the liver to the small intestine is blocked, due to sclerosis or stenosis of extrahepatic bile ducts. The first signs, which appear in the first few weeks of life, are jaundice and acholic stools. The condition was fatal until the introduction of the Kasai hepatoportoenterostomy, which restores bile flow, in 1955 (Jimenez-Rivera and others, 2013). The prevalence of biliary atresia is 5–10 per 100,000 births, and it is the number one reason for pediatric liver transplant (Schreiber and Butler, 2017). More than 70% of individuals with biliary atresia ultimately require a liver transplant (Arnon and others, 2016; McDiarmid and others, 2004).

Etiologies of biliary atresia are not well-described but likely to be multifactorial with genetic and environmental contributors (Ningappa and others, 2015). One hypothesis is that an exaggerated immune response or infection lead to inflammation and obliteration of the biliary tree. Other studies suggest that biliary atresia may result from genetic susceptibilities that affect structural development (Ningappa and others, 2015). Onset may occur as early 11 to 13 weeks gestation, when the bile ducts begin to develop (Asai and others, 2015), but timing likely varies depending on the underlying etiology. Epidemiologic studies are conflicting with respect to risk factors such as maternal age, race-ethnicity, age, infection and seasonality (Caton and others, 2004; Jimenez-Rivera and others, 2013; The and others, 2007). We are aware of one previous study that examined whether biliary atresia risk was associated with maternal nutritional factors. It used data from the National Birth Defects Prevention Study (NBDPS) and observed an association with maternal intake of several nutrients, but risk estimates tended to be imprecise (The and others, 2007).

The current analysis is a follow-up of the previous NBDPS analysis of maternal nutrient intake, which included 62 cases who were born from 1997–2002 (The and others, 2007). The current study includes data from 152 NBDPS cases classified as non-syndromic, born 1997–2011. Given prior findings regarding the potential contribution of the inflammatory and immune response to biliary atresia, we examined nutrients that may impact these pathways (e.g., selected anti-oxidants, B vitamins, and minerals). Given the uncertain etiology of biliary atresia, we also examined nutrients that are emphasized during pregnancy (e.g., protein, calcium, iron); glycemic index due to known associations of various birth defects with maternal glycemic control; and diet quality, a summary indicator of dietary intake. Our hypothesis was that higher intake would be associated with lower risk, with the exception of glycemic index, which we hypothesized would be associated with higher risk.

METHODS

The NBDPS is a multi-site population-based case-control study of major congenital malformations that collected data from women with estimated dates of delivery from 10/1/1997 to 12/31/2011. Study methods have been published (Reefhuis and others, 2015; Yoon and others, 2001). This analysis includes cases with biliary atresia, extrahepatic or not otherwise specified, diagnosed and confirmed postnatally by ultrasound, CT, MRI, hepatobiliary iminodiacetic acid (HIDA) scan, surgery, or autopsy (intrahepatic cases were not eligible). Cases with known or strongly suspected single gene conditions, chromosome abnormalities, multiple situs anomalies or heterotaxy were ineligible. Each eligible case was reviewed by a clinical geneticist and classified as isolated (i.e., no other major anomalies unrelated to biliary atresia) or non-isolated (i.e., at least one additional major unrelated anomaly). Each NBDPS site randomly selected as controls approximately 100 liveborn infants without major birth defects per study year from birth certificates (Arkansas, Georgia, Iowa, Massachusetts, New Jersey, North Carolina, Utah) or birth hospitals (California, New York, Texas) to represent the population from which cases were derived.

Maternal interviews were conducted using a standardized, computer-based questionnaire, primarily by telephone, in English or Spanish, from 6 weeks to 24 months after the estimated date of delivery. Participation among eligible mothers was 68% for biliary atresia cases and 64% for controls. Interview data were available for mothers of 202 cases and 11,829 controls. Median time from date of delivery to interview was 13 months for cases (interquartile range 9–17 months) and 7 months for controls (interquartile range 5–12 months). We excluded the 30 non-isolated cases from analyses, leaving 172 isolated cases (all were live births).

Average intake of foods was assessed by a validated 58-item food frequency questionnaire (FFQ) developed by Willett and colleagues for The Nurses' Health Study.(Willett and others, 1985) Women reported their average intake during the year before they became pregnant. Intake of breakfast cereals, sodas, and food supplements were assessed by separate, more detailed questions, which covered intake during the three months before pregnancy. Food supplements (e.g., powdered drink supplements) were not included in nutrient calculations, since nutrient data were not available for many of these products; 12% of women consumed these types of products. The USDA nutrient database version 27 was used to obtain nutrient intake values (USDA-Agricultural-Research-Service, 2012). We excluded women whose energy intakes were less than 500 kcal or greater than 5,000 kcal or had more than one food item missing from the food frequency questionnaire.

We selected the following nutritional factors for study: alpha- and beta-carotene, betaine, calcium, choline, folate, glycemic index, iron, lutein, methionine, niacin, protein, riboflavin, thiamin, vitamins A, B_6, B_{12}, C, E, zinc, and a diet quality index (DQI). The DQI reflects pregnancy-specific nutritional recommendations and was based on a previously validated index (Bodnar and Siega-Riz, 2002; Haines and others, 1999) that we adapted to apply to the NBDPS FFQ (Carmichael and others, 2012). It is the summary score of six positively scored components (grains, vegetables, fruits, folate, iron, and calcium) and two negatively scored components (intake of sweets, and percent of calories from fat). For each woman, each

component was scored from zero to three based on quartiles of the distribution among control mothers, and then the component scores were summed to obtain the final value.

Odds ratios (OR) and 95% confidence intervals (CI) were generated from logistic regression models to reflect the association of each nutritional factor with biliary atresia. Nutritional factors were entered as continuous variables. ORs were adjusted for energy intake only (kcals) and then for energy intake as well as several covariates previously shown to be associated with biliary atresia or nutrient intake: maternal race-ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, other); education (<,=,or >high school); age (years); pre-pregnancy BMI (kg/m^2); any versus no intake of folic acid-containing vitamin/mineral supplements during the periconceptional period (the month before or first three months after conception); and conception during summer months (June-August) versus other months (based on preliminary findings of this comparison reflecting the season that was associated with biliary atresia in this dataset). We also ran these two sets of models after adding a quadratic (squared) term for each respective nutrient, as a test for non-linearity of its association with biliary atresia. The quadratic terms for seven nutrients had p-values <0.10: choline, niacin, vitamin E, calcium, zinc, protein, and methionine. For these nutrients, we present results derived from models that included a quadratic term. For each final model, we present an OR that reflects the difference between the 75th and 25th percentile of the nutrient, based on its distribution among controls. Energy-adjusted analyses included the 152 cases (of 172) and 11,112 controls (of 11,829) who had complete nutritional data. Analyses adjusted for additional covariates included 141 cases and 10,462 controls with complete data. We also performed analyses after including non-isolated cases.

RESULTS

A majority of subjects were non-Hispanic white, had education greater than high school, and took vitamin supplements (Table 1). Cases were more likely than controls to be born preterm or low birthweight. The OR for any versus no periconceptional vitamin use, adjusted for other covariates, was 1.26 (95% CI 0.71–2.24).

The 95% confidence intervals for most nutrients included 1.00. Exceptions were energy-adjusted ORs for calcium (0.63), protein (OR 0.65), riboflavin (0.71), and the diet quality index (0.69) and the fully adjusted ORs for calcium (0.68) and vitamin E (0.72). ORs that were adjusted for all covariates were relatively similar to ORs adjusted only for energy intake but tended to be slightly closer to one. The energy-adjusted OR for glycemic index suggested that higher values were associated with increased risk (OR 1.25, 95% CI 1.00–1.57). Results were similar when non-isolated cases were included (data not shown).

As noted above, the quadratic term had a p-value <0.10 for seven nutrients, and for these nutrients we derived ORs from models that included the quadratic terms. Upon closer examination, the portion of the curves that included the 25th to 75th percentile values appeared relatively linear; the non-linear part of the curves appeared to be driven by extremes on the upper end of the distributions (data not shown).

DISCUSSION

We examined the association of maternal intake of a variety of nutrients with risk of biliary atresia. For most of the studied nutrients, the odds ratios were in the hypothesized direction (i.e., less than 1.0), but only a few had confidence intervals that excluded 1.0. Maternal intake of vitamin/mineral supplements was not associated with odds of biliary atresia. We hypothesized that one nutritional factor – glycemic index – would be associated with increased risk; its odds ratio was 1.2 (95% CI 1.0–1.6). Thus, in general, the analysis provides modest support for a contribution of maternal dietary intake to risk of biliary atresia.

We are aware of one prior investigation of the contribution of women's periconceptional nutrient intake and biliary atresia in offspring. The prior study by The et al. also used NBDPS data, but for fewer cases and birth years than the current analysis (The and others, 2007). For each nutrient, they collapsed quartiles into two groups based on their initial quartile-specific findings for each nutrient; thus, the presented comparisons varied across the nutrients. In contrast, we specified nutrients as continuous in our models, and we included quadratic terms to assess non-linearity of associations; this approach has the advantages of being consistent, maximizing power, and minimizing misclassification. We examined a somewhat different set of nutrients, given that *a priori*, we chose nutrients that may protect against inflammation or infection and that are related to nutritional guidelines for pregnant women. Both analyses found reduced risks associated with vitamin E and calcium, one difference being that in the prior study, the CI included 1.0 after adjustment for covariates, whereas in our study it excluded 1.0. Neither study found associations with several other nutrients (folate, vitamin B_6, iron, niacin, riboflavin, thiamin, vitamin C) or intake of vitamin supplements. Results suggested that higher glycemic index was associated with increased risk; prior reports have suggested an association of biliary atresia with maternal diabetes but were limited by their design or imprecision (Correa and others, 2008; Herrmann and others, 2004).

Strengths of our study include its population-based design, careful case review, and detailed maternal interview. The sample size is also a strength, given the rarity of biliary atresia. As noted, we considered the possibility of non-linear associations by including quadratic terms in the models. However, for the nutrient models that included these terms, associations were relatively linear except for the upper extremes of the distribution, which apply to very few women and could reflect reporting errors. We adjusted for a variety of covariates; we did not adjust for any markers of maternal infection, given that a variety of such markers were not associated with risk in the prior NBDPS analysis of biliary atresia, and they were non-specific (The and others, 2007). Recall bias is a concern of case-control studies. It is unlikely that our results would be driven by recall bias, given the complexity of food frequency questionnaires and the lack of prior known associations of nutrition with biliary atresia. Participation was <70%, which raises concerns about selection bias; prior analyses suggest that participants and non-participants had similar demographic characteristics (Cogswell and others, 2009). Time to interview was longer for cases than controls; prior analyses suggested that time to interview did not appear to be associated with a variety of exposures, but dietary intake was not evaluated (Tinker and others, 2013). We examined 22 nutritional factors; this

amounts to a modest number of comparisons but type II error remains a potential alternative explanation for our few positive findings. In addition, nutrients are consumed together and thus correlated, which hampers our ability to identify independent effects (e.g., correlations among controls of the 5 nutritional factors with significant associations – calcium, protein, riboflavin, diet quality and vitamin E – ranged from 0.51 to 0.85); examination of the diet quality index represents one attempt to address this limitation.

In conclusion, this study provides evidence for an association of biliary atresia with maternal dietary intake of some nutrients but is limited by its relatively small number of cases. Despite our limited findings, studies should continue to investigate maternal nutrition when possible, given that it is potentially related to pathways that have been proposed to contribute to biliary atresia. Understanding mechanisms will improve our ability to identify causes and effectively prevent, screen and treat biliary atresia.

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References

Arnon R, Annunziato RA, D'Amelio G, Chu J, Shneider BL. Liver Transplantation for Biliary Atresia: Is There a Difference in Outcome for Infants? J Pediatr Gastroenterol Nutr. 2016; 62(2):220–225. [PubMed: 26418214]

Asai A, Miethke A, Bezerra JA. Pathogenesis of biliary atresia: defining biology to understand clinical phenotypes. Nat Rev Gastroenterol Hepatol. 2015; 12(6):342–352. [PubMed: 26008129]

Bodnar LM, Siega-Riz AM. A Diet Quality Index for Pregnancy detects variation in diet and differences by sociodemographic factors. Public Health Nutr. 2002; 5(6):801–809. [PubMed: 12570888]

Carmichael SL, Yang W, Feldkamp ML, Munger RG, Siega-Riz AM, Botto LD, Shaw G. National Birth Defects Prevention S. Reduced risks of neural tube defects and orofacial clefts with higher diet quality. Archives Pediatr Adol Med. 2012; 166(2):121–126.

Caton AR, Druschel CM, McNutt LA. The epidemiology of extrahepatic biliary atresia in New York State, 1983–98. Paediatr Perinat Epidemiol. 2004; 18(2):97–105. [PubMed: 14996248]

Cogswell ME, Bitsko RH, Anderka M, Caton AR, Feldkamp ML, Hockett Sherlock SM, Meyer RE, Ramadhani T, Robbins JM, Shaw GM, Mathews TJ, Royle M, Reefhuis J. Control selection and participation in an ongoing, population-based, case-control study of birth defects: the National Birth Defects Prevention Study. Am J Epidemiol. 2009; 170(8):975–985. [PubMed: 19736223]

Correa A, Gilboa SM, Besser LM. Diabetes mellitus and birth defects. Am J Obstet Gynecol. 2008; 199:231–239.

Haines PS, Siega-Riz AM, Popkin BM. The diet quality index revised: a measurement instrument for populations. J Am Diet Assoc. 1999; 99:697–704. [PubMed: 10361532]

Herrmann J, Brauer M, Scheer I, Barthlen W, Buhrer C. Extrahepatic biliary atresia and caudal regression syndrome in an infant of a diabetic mother. J Pediatr Surg. 2004; 39(1):E20–22.

Jimenez-Rivera C, Jolin-Dahel KS, Fortinsky KJ, Gozdyra P, Benchimol EI. International incidence and outcomes of biliary atresia. J Pediatr Gastroenterol Nutr. 2013; 56(4):344–354. [PubMed: 23263590]

McDiarmid SV, Anand R, Lindblad AS. Studies of Pediatric Liver Transplantatio: 2002 update. An overview of demographics, indications, timing, and immunosuppressive practices in pediatric liver transplantation in the United States and Canada. Pediatric transplantation. 2004; 8(3):284–294. [PubMed: 15176967]

Ningappa M, Min J, Higgs BW, Ashokkumar C, Ranganathan S, Sindhi R. Genome-wide association studies in biliary atresia. Wiley Interdiscip Rev Syst Biol Med. 2015; 7(5):267–273. [PubMed: 25963027]

Reefhuis J, Gilboa SM, Anderka M, Browne ML, Feldkamp ML, Hobbs CA, Jenkins MM, Langlois PH, Newsome KB, Olshan AF, Romitti PA, Shapira SK, Shaw GM, Tinker SC, Honein MA. The National Birth Defects Prevention Study: A review of the methods. Birth Defects Res A Clin Mol Teratol. 2015; 103:656–669. [PubMed: 26033852]

Schreiber RA, Butler A. Screening for biliary atresia: it's in the cards. Can Fam Physician. 2017; 63(6): 424–425. [PubMed: 28615387]

The NS, Honein MA, Caton AR, Moore CA, Siega-Riz AM, Druschel CM. National Birth Defects Prevention S. Risk factors for isolated biliary atresia, National Birth Defects Prevention Study, 1997–2002. Am J Med Genet A. 2007; 143A(19):2274–2284. [PubMed: 17726689]

Tinker SC, Gibbs C, Strickland MJ, Devine OJ, Crider KS, Werler MM, Anderka MT, Reefhuis J. National Birth Defects Prevention S. Impact of time to maternal interview on interview responses in the National Birth Defects Prevention Study. Am J Epidemiol. 2013; 177(11):1225–1235. [PubMed: 23645625]

USDA-Agricultural-Research-Service. Nutrient Data Laboratory Home Page. 2012. USDA National Nutrient Database for Standard Reference, Release 25.

Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol. 1985; 122(1):51–65. [PubMed: 4014201]

Yoon PW, Rasmussen SA, Lynberg MC, Moore CA, Anderka M, Carmichael SL, Costa P, Druschel C, Hobbs CA, Romitti PA, Langlois PH, Edmonds LD. The National Birth Defect Prevention Study. Public health reports (Washington, DC : 1974). 2001; 116(Suppl 2):32–40.

Table 1

Characteristics of cases with biliary atresia and controls without major birth defects.

	Percent	
Maternal characteristics	**Cases (n=152)**	**Controls (n=11,112)**
Race-ethnicity		
Non-Hispanic white	53	59
Non-Hispanic black	18	11
Black	20	24
Other	9	6
Missing	0	<1
Education		
< High school	17	16
= High school	20	24
> High school	61	60
Missing	1	1
Periconceptional intake of folic acid-containing supplements [1]		
No	9	12
Yes	90	87
Missing	1	1
Season at conception		
Summer	19	25
Other	81	75
Missing	0	0
	Mean (SD)	Mean (SD)
Body mass index (kg/m^2) [2]	25 (6)	25 (6)
Age (years) [2]	27 (6)	28 (6)
Infant characteristics	Percent	Percent
Gestational age at delivery		
Preterm (<37 weeks)	17	9
Term (37 or more weeks)	83	91
Missing	0	0
Birthweight		
Low (<2500 gm)	9	6
No low (2500 gm or more	89	93
Missing	1	1

Maternal characteristics	Percent	
	Cases (n=152)	Controls (n=11,112)
Birthweight for gestational age		
Term, normal birthweight	77	88
Term, low birthweight	4	2
Preterm, normal birthweight	12	5
Preterm, low birthweight	5	4
Missing	1	1
Sex		
Male	48	51
Female	52	49
Missing	0	<1
Plurality		
Singleton	96	97
Higher order birth	4	3
Missing	0	<1

[1] Periconceptional refers to the month before conception through three months after conception.

[2] 8 cases and 449 controls had missing data on BMI; none had missing maternal age.

Table 2

Odds ratios for the association of biliary atresia with maternal intake of selected nutrients and diet quality, reflecting odds for the 75th versus 25th percentile of intake, National Birth Defects Prevention Study, 1997–2011.

Nutrient (unit change from the 75th to 25th percentile):	Odds ratio (95% CI) [1]	
	Energy-adjusted	Adjusted for energy intake and covariates
Alpha-carotene (698.0 ug)	0.96 (0.82–1.14)	1.02 (0.88–1.18)
Beta-carotene (2642.1 ug)	1.00 (0.84–1.19)	1.04 (0.88–1.23)
Betaine (60.4)	0.89 (0.70–1.13)	0.90 (0.70–1.14)
Calcium[2] (546.3 mg)	**0.63 (0.47–0.87)**	**0.68 (0.49–0.95)**
Choline[2] (173.3 ug)	0.73 (0.51–1.04)	0.75 (0.52–1.09)
Folate (344.9 ug DFE)	0.89 (0.71–1.13)	0.89 (0.70–1.12)
Glycemic index (6.5 units)	1.25 (1.00–1.57)	1.22 (0.95–1.57)
Iron (8.9 mg)	0.94 (0.74–1.20)	0.95 (0.75–1.21)
Lutein (1527.9 ug)	1.04 (0.94–1.16)	1.03 (0.93–1.15)
Methionine[2] (0.8 gm)	0.71 (0.50–1.00)	0.76 (0.53–1.09)
Niacin[2] (10.3 mg)	0.85 (0.62–1.17)	0.85 (0.61–1.19)
Protein[2] (33.9 gm)	**0.65 (0.45–0.94)**	0.69 (0.46–1.03)
Riboflavin (1.2 mg)	**0.71 (0.51–0.99)**	0.77 (0.55–1.09)
Thiamin (0.7 mg)	0.94 (0.71–1.24)	0.92 (0.69–1.23)
Vitamin A (509.1 ug RAE)	0.91 (0.73–1.12)	0.92 (0.75–1.14)
Vitamin B_6 (1.2 ug)	0.85 (0.62–1.14)	0.86 (0.63–1.16)
Vitamin B_{12} (3.7 ug)	0.88 (0.72–1.08)	0.87 (0.70–1.08)
Vitamin C (98.4 mg)	0.82 (0.64–1.04)	0.79 (0.61–1.03)
Vitamin E[2] (3.9 mg)	0.76 (0.57–1.01)	**0.72 (0.53–0.96)**
Zinc[2] (6.0 ug)	0.74 (0.52–1.04)	0.77 (0.54–1.10)
Diet quality index (8 units)	**0.69 (0.51–0.95)**	0.75 (0.54–1.05)

DFE = dietary folate equivalents, RAE = retinol activity equivalents

[1] 152 cases and 11,112 controls were included in the energy-adjusted models and 141 cases and 10,462 controls in models adjusted for all covariates, which included maternal race-ethnicity, body mass index (kg/m^2), age (years), education, intake of folic acid-containing supplements during the month before pregnancy or first trimester, and conception during summer months versus other months. ORs reflect the difference in odds for intake at the 75th versus 25th percentile of intake, based on the distribution among the controls. ORs are bolded if the CI excluded 1.00 after rounding.

[2] The quadratic terms for protein, methionine, niacin, choline, vitamin E, calcium and zinc had $p<0.10$ in energy-adjusted and fully adjusted models; ORs for these nutrients were derived from models that included quadratic terms.