Long-Term Impact of Neonatal Breastfeeding on Childhood Adiposity and Fat Distribution Among Children Exposed to Diabetes In Utero

Tessa L. Crume, PhD1
Lorraine Ogden, PhD1
MaryBeth Malige, BSc1
Shelly Sheffield, MS2
Kimberly J. Bischoff, MSHA2
Robert McDuffie, MD2
Stephen Daniels, MD, PhD3
Richard F. Hamman, MD, DRPH1
Jill M. Norris, PhD1
Dana Dabelea, MD, PhD1

OBJECTIVE—To evaluate whether breastfeeding attenuates increased childhood adiposity associated with exposure to diabetes in utero.

RESEARCH DESIGN AND METHODS—Retrospective cohort study of 89 children exposed to diabetes in utero and 379 unexposed youth with measured BMI, waist circumference, skinfolds, visceral (VAT) and subcutaneous (SAT) abdominal fat. A measure of breast milk months was derived from maternal self-report and used to categorize breastfeeding status as low (<6) and adequate (≥6 breast milk–months). Multiple linear regression was used to model the relationship between exposure to diabetes in utero and offspring adiposity outcomes among youth stratified according to breastfeeding status.

RESULTS—Adequate (vs. low) breastfeeding status was associated with significantly lower BMI, waist circumference, SAT, and VAT at ages 6–13 years. Among youth in the low breastfeeding category, exposure to diabetes in utero was associated with a 1.7 kg/m2 higher BMI (P = 0.03), 5.8 cm higher waist circumference (P = 0.06), 44.6 cm2 higher SAT (P = 0.03), and 0.11 higher ratio of subscapular-to-triceps skinfold ratio (P = 0.008). Among those with adequate breastfeeding in infancy, the effect of prenatal exposure to diabetes on childhood adiposity outcomes was not significant.

CONCLUSIONS—Adequate breastfeeding protects against childhood adiposity and reduces the increased adiposity levels associated with exposure to diabetes in utero. These data provide support for mothers with diabetes during pregnancy to breastfeed their infants in order to reduce the risk of childhood obesity.

Children born to mothers with diabetes during pregnancy have been shown to have a greater prevalence of obesity in childhood, both among populations at high risk for obesity and type 2 diabetes, such as American Indian youth (1,2), as well as among multiethnic populations (3,4). These youth may be “programmed” in utero for later development of obesity by exposure to excess maternal glucose (and other fuels) at a critical period of development (i.e., the fetal overnutrition hypothesis) (5). Infancy has been suggested as another critical period for future obesity risk. Large epidemiologic studies suggest that the early postnatal weeks of life are a critical period for determining levels and distribution of adiposity, a time when breast-fed infants often lose weight and formula-fed infants tend to gain weight (6,7). The relationship between breastfeeding and long-term obesity risk has been extensively studied, and breastfeeding is now promoted as an important prevention strategy with considerable public health benefits. Meta-analyses have estimated an effect size of 13–22% reduced odds for overweight or obesity in childhood and later in life associated with having been breast-fed (8). A dose-response effect was reported by Harder et al. (9) describing a 4% reduction in childhood obesity risk for each additional month of breastfeeding (95% CI [−0.06 to −0.02]). A systematic review and meta-analysis (10) quantified a protective effect on risk for type 2 diabetes in later life for subjects who were breast-fed compared with those formula-fed (7 studies; 76,744 subjects; odds ratio [OR] 0.61 [95% CI 0.44–0.85]; P = 0.003). However, the question of whether breastfeeding may actually reduce the risk of future obesity conferred by fetal overnutrition, as marked by exposure to diabetes in utero, is inconclusive. The objective of this study was to evaluate if breastfeeding is protective against childhood obesity and whether it attenuates the association between exposure to diabetes in utero and adiposity parameters in a multiethnic cohort of youth from Colorado.

RESEARCH DESIGN AND METHODS

Study design and eligibility criteria

This article uses data from a retrospective cohort study, Exploring Perinatal Outcomes among Children (EPOCH), which was conducted in Colorado. Participants were 6–13 years of age; the multiethnic offspring of singleton pregnancies; born at a single hospital in Denver, Colorado, between 1992 and 2002; whose biological mothers were members of the Kaiser Permanente of Colorado Health Plan (KP CO); and who were still KP CO members and living in Colorado over the study period (2006–2009).

For this analysis, eligible participants were children exposed to diabetes in utero (exposed group) and a random sample of children not exposed to diabetes in utero.
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without intrauterine growth restriction (defined as birth weight for gestational age score < the 10th percentile) (unexposed group). Children and their biological mothers were invited for a research visit between January 2006 and October 2009. The study was approved both by the Colorado Multiple Institutional Review Board and Human Participant Protection Program. All participants provided written informed consent, and youth provided written assent.

Exposure definition
Physician-diagnosed maternal diabetes status was ascertained from the KPCO Perinatal database, an electronic database linking the neonatal and perinatal medical records, as described previously. The database contains data that define delivery events for each woman. Gestational diabetes mellitus (GDM) is coded as present if diagnosed through the standard KPCO screening protocol (described below) and absent if screening was negative. At 24–28 weeks, all pregnant women are offered screening with a 1-h 50-g oral glucose tolerance test (OGTT). A value $\geq 140$ mg/dl identifies patients who undergo a 3-h 100-g diagnostic OGTT. GDM is diagnosed when two or more glucose values during the diagnostic OGTT meet or exceed the criteria for a positive test as recommended by the National Diabetes Data Group (11). Exposure to diabetes in utero was defined as the presence of preexistent diabetes or GDM diagnosed during the index pregnancy. In addition, birth weight, gestational age, and maternal pre-pregnancy weight were also obtained from the database.

Breastfeeding status
At the study visits, mothers were queried about breast- and formula-feeding, timing, and the introduction of other solid foods and beverages. Because of the high levels of reported mixed feeding, a measure of breast milk–months was developed that incorporated duration and exclusivity. For exclusively breast-fed infants, duration was equal to the age of the child (months) when breastfeeding was stopped. For infants that were ever fed formula, mothers were asked to classify their infant feeding as formula only, more formula than breast milk, equal breast milk and formula, or more breast milk than formula. Breastfeeding exclusivity was quantified using weights from 0 to 1, with exclusive breastfeeding having a weight of 1 and exclusive formula-feeding having a weight of 0. For infants fed both breast milk and formula, exclusivity was equal to 0.25 for “more formula than breast milk”; 0.50 for “formula and breast milk equally”; and 0.75 for “more breast milk than formula.” The breast milk–months measure incorporated duration and exclusivity to estimate an overall breast milk dose equivalent in months. It was the sum of months of exclusive breastfeeding and the weighted months of mixed breast milk and formula (duration of exclusive breastfeeding [months] + duration of mixed breast- and formula-feeding [months] × exclusivity weight). Breastfeeding status was categorized as low (<6 breast milk–months) and adequate (≥6 breast milk–months) based on American Academy of Pediatrics recommendations (12). Maternal recall of breastfeeding after periods of time spanning between 9 and 20 years has been found to correlate well with infant feeding data obtained from medical records ($r = 0.86$ (13) or collected prospectively ($r = 0.95$) (14).

Measures of childhood adiposity and fat distribution
Childhood height and weight were measured in light clothing and without shoes. Weight was measured to the nearest 0.1 kg using an electronic scale. Height was measured to the nearest 0.1 cm using a portable stadiometer. BMI was calculated as kg/m². Waist circumference was measured to the nearest 1 mm at the midpoint between the lower ribs and the pelvic bone with a metal or fiberglass nonspring-loaded tape measure. Skinfolds were measured in triplicate using Holtain calipers (average: subscapular, ~20 mm below the tip of the scapula; triceps, halfway between the acromion process and the olecranon process). The subscapular-to-triceps skinfold ratio (STR) was calculated to assess regional differences in subcutaneous fat distribution. Magnetic resonance imaging of the abdominal region was used to quantify visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) with a 3T HDx imager (General Electric, Waukesha, WI) by a trained technician. Each study participant was placed supine and a series of T1-weighted coronal images were taken to locate the L4/L5 plane. One axial, 10-mm, T1-weighted image at the umbilicus or L4/L5 vertebra was analyzed to determine SAT and VAT content. The analysis technique used was a modification of the technique of Engelson, where adipose tissue regions were differentiated by their signal intensity and location (i.e., not internal contents of bowel). Images were analyzed by a single reader, blinded to exposure status.

Other measurements
Race/ethnicity was self-reported using 2000 U.S. Census-based questions and categorized as Hispanic (any race), non-Hispanic white, or non-Hispanic African American. Pubertal development was assessed by child self-report with a diagrammatic representation of Tanner staging adapted from Marshall and Tanner (15). Youth were categorized as Tanner <2 (prepubertal) and ≥2 (pubertal). Maternal level of education and total household income were self-reported during the research office visit.

Statistical analysis
All analyses were conducted using SAS v.9.2 (SAS Institute, Cary, NC). Results are presented as means ± SD. Group differences were analyzed using $\chi^2$ test. Multiple linear regression was conducted to examine the association between exposure to maternal diabetes in utero and measures of offspring adiposity (BMI, waist circumference, VAT, SAT, and STR), controlling for potential confounders. In determining the best model for the relationship between adiposity outcomes and in utero diabetes exposure, a significant interaction between age and Tanner stage on VAT was noted ($P = 0.0008$), suggesting that the effect of age on childhood adiposity depends on pubertal development. Because such an effect was reported previously in the literature (16,17), an interaction term between age and Tanner stage was included in our model for all outcomes of interest.

Stratified analyses were conducted to explore the association between exposure to diabetes in utero and childhood adiposity outcomes in offspring with low versus adequate breastfeeding. A formal test for heterogeneity was used to assess whether associations were significantly different.

RESULTS—A total of 89 youth exposed to diabetes in utero (91% with GDM) and 379 unexposed youth participated in the study and had complete data on variables of interest. The mean ± SD age of exposed youth was 9.6 ± 1.7 years and 10.6 ± 1.3 for unexposed youth ($P < 0.0001$) at the study visit. Exposed youth were more likely to be non-Hispanic white or Hispanic ($P = 0.04$), and a larger proportion of exposed youth self-reported a Tanner
stage <2, indicating they were prepubertal (71.1% vs. 50.8%, \( P = 0.0005 \)). Mothers with diabetes during pregnancy were, on average, older (\( P < 0.0001 \)) than mothers whose pregnancies were not complicated by diabetes. Exposed and unexposed offspring were not significantly different in terms of intrauterine growth and socioeconomic factors or infant feeding practices (Table 1).

Table 2 shows the association between breastfeeding status (low vs. adequate) and adiposity outcomes among offspring exposed and unexposed to diabetes in utero. Both exposed and unexposed youth who had adequate breastfeeding (≥6 breast milk–months) had significantly lower BMI, waist circumference, SAT, and VAT at ages 6–13 years than those with <6 breast milk–months. The only adiposity parameter that did not show a statistically significant relationship with breastfeeding was STR (\( P = 0.26 \) and \( P = 0.72 \), respectively, in exposed and unexposed participants), though the pattern was similar.

Figure 1 shows the associations between exposure to diabetes in utero and adiposity outcomes stratified by breastfeeding status and adjusted for age, sex, Tanner stage, and Tanner by age interaction. Among adolescents with low breastfeeding status (<6 breast milk–months), exposure to diabetes in utero was associated with a 1.7 kg/m\(^2\) higher BMI (\( P = 0.03 \)), 5.8 cm higher waist circumference (\( P = 0.008 \)); 6.1 cm\(^2\) higher VAT (\( P = 0.06 \)); 44.6 cm\(^2\) higher SAT (\( P = 0.03 \)); and 0.11 higher STR (\( P = 0.008 \)). The association between exposure to diabetes in utero and the adiposity parameters was substantially reduced and not significant for adolescents with adequate breastfeeding in infancy (≥6 breast milk–months) with a 0.7 kg/m\(^2\) lower BMI (\( P = 0.4 \)); 2.7 cm higher waist circumference (\( P = 0.1 \)); 2.1 cm\(^2\) higher VAT (\( P = 0.4 \)); 23.4 cm\(^2\) higher SAT (\( P = 0.11 \)); and 0.05 higher STR (\( P = 0.14 \)) among exposed versus unexposed children.

**CONCLUSIONS**—We found that adequate breastfeeding (≥6 breast milk–months) was associated with lower BMI, waist circumference, and SAT levels in a multiethnic population of youth 6–13 years of age from Colorado. Moreover, our study provides novel evidence that the effect of exposure to diabetes in utero on childhood adiposity parameters is substantially attenuated by breastfeeding, such that the obesity outcomes in exposed youth who were adequately breast-fed were similar to those of unexposed youth. Our data suggest that breastfeeding promotion may be an effective strategy for reducing the increased risk of childhood obesity in the offspring of mothers with diabetes during pregnancy.

The current literature on the impact of breastfeeding for the offspring of diabetic women is inconclusive. Plagemann et al. (18) suggested an adverse effect of breastfeeding during the first 7 days of life on relative weight at 2 years of age among offspring of women with type 1 diabetes and GDM. However, a follow-up of this study was conducted by Rodekamp et al. (19) with an extended assessment of breastfeeding exposure beyond the first week of life. The researchers found that neither dose of breast milk nor duration of breastfeeding among offspring of type 1 diabetic women was associated with increased risk of overweight or impaired glucose tolerance at 2 years of age. In another study, Kerssen et al. (20) showed no effect of breast milk, formula, or mixed feeding on the weight or BMI of offspring.
exposed to type 1 diabetes during pregnancy at 1 year of age. In contrast, among 15,253 offspring of the 1989 Nurses’ Health Study II, Mayer-Davis et al. (21) reported a protective OR of 0.63 (95% CI 0.50–0.78) for overweight at 12 years of age for breastfeeding duration of ≥9 versus <9 months. OR for overweight among offspring exposed to GDM in utero who were exclusively breast-fed compared with those exclusively formula-fed was 0.62 (0.24–1.60). Among Pima Indians, Pettitt and Knowler (22) found a substantial reduction (30.1% vs. 43.6%) in type 2 diabetes among the offspring exposed to diabetes in utero if they were breast-fed for at least 2 months compared with those who were bottle-fed. A similar reduction was observed among Pima offspring not exposed to in utero diabetes (6.9 and 11.9% among offspring of nondiabetic women who were breast-fed and bottle-fed, respectively).

The early postnatal period may represent a critical period for the future obesity risk in childhood (23) and adult life (6). The macronutrient composition of breast milk (i.e., protein, fat, carbohydrate) and bioactive substances not present in formula may influence metabolic programming and regulation of body fatness and growth rate. Higher insulin levels (24) and lower leptin levels (25) have been reported in formula-fed infants compared with breast-fed babies. The effect of breastfeeding on infant growth may be an important determinant of early life programming for future obesity and chronic disease, especially for the offspring of diabetic pregnancies.

Our study adds to the limited body of evidence by suggesting that breastfeeding attenuates the unfavorable effects on childhood adiposity parameters conferred by exposure to maternal diabetes. Importantly, all measures of adiposity were influenced including the more sensitive VAT and SAT. The mechanisms that trigger adipose tissue deposition in specific locations at different periods of fetal development or in childhood remain unclear. Identification of strategies to alter the long-term development of fat deposition and accumulation are necessary to minimize the significant increased morbidity risk associated with childhood obesity. Fetal life and early infancy both represent critical periods when obesity begins and may be effectively minimized by targeted prevention strategies.

Our study had some limitations. An a priori power calculation suggested that, with 100 exposed individuals, our study had 80% power to detect a difference in the effect of exposure to diabetes in utero on childhood BMI according to breastfeeding status (an interaction) of 0.4 BMI units. The observed difference in our study was 0.5 BMI units; however, the difference was not statistically significant, possibly because of the slightly smaller achieved sample of exposed individuals. This suggests that we had limited power to detect a statistically significant effect of breastfeeding in modifying the association between exposure to diabetes in utero and childhood adiposity. Nevertheless, our findings of an attenuation of the effect of in utero exposure on childhood adiposity by breastfeeding was robust and consistent across various measures of adiposity including BMI, waist circumference, SAT, VAT, and fat distribution. Similar effects were noted regardless of how our breastfeeding variable was defined (i.e., breastfeeding yes/no, duration in months, etc.; data not shown). Moreover, our study had several important strengths including state-of-the-art assessment of childhood adiposity and fat distribution, a validated

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Table 2—Association between breast milk–months (<6 and ≥6 breast milk–months) and adiposity parameters at age 6–13 years for youth exposed and not exposed to maternal diabetes in utero

| Breast milk–months | Unexposed to diabetes in utero | Exposed to diabetes in utero |
|--------------------|-------------------------------|-------------------------------|
|                    | ≥6 m  | <6 m | P   | ≥6 m  | <6 m | P   |
| n                  | 174   | 205  |    | 41    | 48   |    |
| BMI (kg/m²)        | 18.6  | 19.6 | 0.02| 18.0  | 20.1 | 0.05|
| Waist circumference (cm) | 64.9  | 67.2 | 0.05| 62.4  | 68.4 | 0.03|
| SAT (cm²)          | 114.7 | 137.9| 0.03| 97.9  | 152.3| 0.02|
| VAT (cm²)          | 22.4  | 26.3 | 0.03| 21.5  | 29.2 | 0.09|
| STR                | 0.76  | 0.77 | 0.72| 0.76  | 0.83 | 0.26|

Means from multiple linear regression model adjusted for age, sex, race/ethnicity, Tanner stage, and age × Tanner stage interaction. m, months.

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Figure 1—A–E: Effect of exposure to diabetes in utero on mean levels of childhood adiposity parameters. Stratified by breastfeeding (BF) status, data are adjusted for age, sex, race/ethnicity, Tanner stage, and age × Tanner stage interaction. A: BMI: P = 0.03 for youth with low breastfeeding; P = 0.4 for youth with adequate breastfeeding. B: Waist circumference (WC): P = 0.008 for youth with low breastfeeding; P = 0.10 for youth with adequate breastfeeding. C: VAT: P = 0.06 for youth with low breastfeeding; P = 0.40 for youth with adequate breastfeeding. D: SAT: P = 0.03 for youth with low breastfeeding; P = 0.011 for youth with adequate breastfeeding. E: STR: P = 0.008 for youth with low breastfeeding; P = 0.14 for youth with adequate breastfeeding.
exposure assessment, and an assessment of breast milk dose that incorporated breastfeeding exclusivity and duration.

In conclusion, our study found no deleterious effects of breastfeeding among a diverse group of children exposed to diabetes in utero. In contrast, we suggest that breastfeeding may be protective against the increased childhood adiposity associated with intrauterine diabetes exposure. Further work is needed to confirm this finding in larger populations, and to determine if the reductions in adiposity continue into adulthood.

Acknowledgments—This work was supported by National Institutes of Health Grant R01-DK068001 (principal investigator D.D.). The study sponsor had no role in the study. No potential conflicts of interest relevant to this article were reported.

T.L.C. researched data and wrote the manuscript. L.O. reviewed and edited the manuscript and advised on analysis. M.M. researched data. L.O. reviewed and edited the manuscript. J.M.N. reviewed and edited the manuscript. R.F.H. reviewed the manuscript. S.D. reviewed the manuscript. S.S. researched data. K.J.B. researched data. M.M. researched data. L.O. reviewed and edited the manuscript. This work was supported by National Institutes of Health Grant R01-DK068001 (principal investigator D.D.). The study sponsor had no role in the study. No potential conflicts of interest relevant to this article were reported.

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