Selective Protection Against Extremes in Childhood Body Size, Abdominal Fat Deposition, and Fat Patterning in Breastfed Children

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Objective: To determine whether the effect of breastfeeding on childhood measures of adiposity differs across percentiles of childhood body mass index (BMI), subcutaneous (SAT) and visceral (VAT) adipose tissue deposition, ratio of subcutaneous to triceps skinfold (STR), and intramyocellular lipid accumulation (IMCL).

Design: Retrospective cohort.

Setting: Denver, Colorado.

Participants: Four hundred forty-two children and adolescents aged 6 to 13 years participating in the Exploring Perinatal Outcomes Among Children study (EPOCH) with material recall of infant diet.

Main Exposure: Adequate (≥6 breast milk–months) vs low (<6 breast milk–months) neonatal breastfeeding status.

Main Outcome Measures: Mean and percentile differences in BMI, SAT, VAT, STR, and IMCL between children who had adequate compared with low levels of breastfeeding.

Results: No significant differences in mean levels of childhood adiposity levels between adequate and low breastfeeding status were detected using linear regression models. However, in quantile regression models, adequate breastfeeding was associated with lower levels of adiposity levels for those in the upper percentiles (>60th percentile for VAT, 85th and 95th percentiles for BMI, and 95th percentiles for SAT and STR) and a null effect for those at the 50th percentile or lower. These effects were independent of sociodemographic, perinatal, and current lifestyle factors. We found no relationship between breastfeeding and IMCL at any percentile of the distribution.

Conclusions: Rather than shifting the entire distribution of adiposity-related measures in childhood, breastfeeding selectively protects against extremes in body size and fat deposition, reinforcing the American Academy of Pediatrics recommendation for 6 months of exclusive breastfeeding as the optimal infant diet.

uous (SAT) adipose tissue; fat patterning, assessed as the ratio of subscapular to triceps skinfold (STR); and ectopic fat deposition based on intramyocellular lipid (IMCL) accumulation.

METHODS

The Exploring Perinatal Outcomes Among Children study (EPOCH) is a retrospective cohort study conducted in Colorado. Participants included a multiethnic population of children and adolescents (hereinafter referred to as children) aged 6 to 13 years who were the offspring of singleton pregnancies and born at a single hospital in Denver during the period from January 1, 1992, through December 31, 2002. Their biological mothers were members of Kaiser Permanente of Colorado Health Plan at the time of birth and were still members and living in Colorado during the study period. Children and their biological mothers were invited for a research visit from January 1, 2006, through December 1, 2009. The present analysis includes 442 EPOCH participants born to mothers who did not have diabetes mellitus during the pregnancy.

MEASURES OF CHILDHOOD BODY SIZE 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. Body mass index was calculated as weight in kilograms divided by height in meters squared. Magnetic resonance imaging of the abdominal region was used to quantify VAT and SAT with a 3-T system (HDx imager; General Electric) 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 vertebrae was analyzed to determine SAT and VAT content. A detailed description of the analysis technique has been described elsewhere. Images were analyzed by a single reader (A.L.S.) blinded to exposure status. Accumulation of IMCL was assessed by magnetic resonance spectroscopy. Each subject was positioned to the midcalf area using a T1-weighted image as a localizer, and homogeneous muscle regions were selected for measurement. The spectroscopy acquisition was performed using the point-resolved spectroscopy pulse sequence (repetition time, 2000 milliseconds; echo time, 100 milliseconds) with the creatine peak at 3.0 ppm used for an internal reference. Water suppression was not used, so the IMCL concentration could be scaled to the basis set water peak and therefore normalized. Although not given in absolute units, IMCL can therefore be compared across the subjects studied. Spectra images were analyzed using the spectroscopy analysis package (SAGE; General Electric).

BREASTFEEDING STATUS

At the study visit, mothers were queried about breastfeeding and formula feeding and the timing and introduction of other solid foods and beverages. Because of high levels of reported mixed feeding, a measure of breast milk–months was developed that incorporated duration and exclusivity. For all infants who were ever breastfed, duration was equal to the age of the child (in months) when breastfeeding was stopped entirely; therefore, duration included periods of mixed breastfeeding and formula feeding. For infants ever fed formula, mothers were asked to classify their infant feeding as formula only, more formula than breast milk, equal amounts of breast milk and formula, or more breast milk than formula. Breastfeeding exclusivity was quantified using weights from 0 and 1, with exclusive breastfeeding having a weight of 1 and exclusive formula feeding having a weight of 0. For infants fed 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 as the sum of months of exclusive breastfeeding and the weighted months of mixed breast milk and formula in the following equation:

\[
\text{Breast milk–months} = \text{Duration of Exclusive Breastfeeding (in Months)} + \frac{\text{Duration of Mixed Breastfeeding and Formula Feeding (in Months)}}{2} \times \text{Exclusivity Weight.}
\]

Breast milk–months were categorized as low (<6 months) and adequate breastfeeding status (≥6 months) based on recommendations by the American Academy of Pediatrics.13

OTHER MEASUREMENTS

Race/ethnicity was self-reported using 2000 US 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.14 Youth were categorized as being at a Tanner stage of less than 2 (prepubertal) or 2 or more (pubertal). Maternal level of education and total household income were self-reported at the study visit. Children’s total energy intake (kilocalories per day) was assessed using the Block Kid’s Food Questionnaire.15 Self-reported sedentary and nonsedentary key activities performed during the previous 3 days were queried using a 3-day physical activity recall questionnaire.16 Each 30-minute block of activity was assigned a metabolic equivalent variable to accommodate the energy expenditure. Results were reported as a percentage score reflecting the extent to which the number of 30-minute blocks of moderate-to-vigorous and vigorous physical activity reported during the 3 days met the standard of at least 1 hour of moderate-to-vigorous physical activity per day17 and 20 minutes of vigorous physical activity per day.18 Maternal prepregnancy BMI was calculated from the Kaiser Permanente of Colorado–measured weight before the last menstrual cycle preceding pregnancy and height collected at the in-person research visit.

STATISTICAL ANALYSIS

Multiple regression techniques were used to model the effect of breastfeeding on the following adiposity outcomes of interest: BMI, SAT, VAT, STR, and IMCL. Linear regression was used to estimate the conditional mean difference in adiposity levels between children with low and adequate breastfeeding patterns. Quantile regression was used to estimate the conditional median differences and differences at the 5th, 15th, 30th, 40th, 50th, 60th, 70th, 85th, and 95th percentiles of adiposity levels. Our multivariable model controlled for the effects of age, sex, race/ethnicity, Tanner stage, daily caloric intake, and physical activity levels and for maternal factors, including smoking during pregnancy, income, level of education, and prepregnancy BMI. In addition, an interaction term between age and Tanner stage found to be significant in our data set and in other studies19,20 was included for all outcomes of interest. All calculations were performed in the open-source statistical envi-
environment \( R^2 \) using the Quantreg package. \(^{11}\) Standard errors and confidence limits were obtained for the regression coefficients using the sparsity method that assumes errors are independently and identically distributed. The asymptotic method was used to compute the covariance and correlation matrices of the estimated variables.

**ETHICAL CONSIDERATIONS**

The protocol was approved by the multiple institutional review board and Human Participant Protection Program. Written informed consent was obtained from all participants, and youth provided written assent. Confidentiality was ensured; mothers and their children were advised that their participation was voluntary and that they could withdraw at any time.

**RESULTS**

Table 1 shows the characteristics of the study participants, of whom 48.9% were male, 51.4% were prepubertal, 47.5% were non-Hispanic white, 43.2% were Hispanic, and 9.3% were African American. The mean self-reported total daily calories and percentage of calories from fat were within recommend levels, and 82.8% of children reported a level of moderate to vigorous and vigorous physical activity that meets recommended levels. \(^{17,18}\) More than 99% of the mothers reported having at least a high school education and 78.7% reported a total household income of more than $50,000 per year. Average maternal BMI was 25.6, and 6.1% of the mothers self-reported smoking during pregnancy. Table 2 shows the correlations between various measures of adiposity in our study cohort. Although all the correlations were statistically significant \((P < .001)\), the correlations between current BMI and VAT and STR were somewhat weaker \((r = 0.67 \text{ and } r = 0.51, \text{respectively})\) than those between BMI and SAT \((r = 0.93)\). We found only a weak correlation between IMCL and measures of adiposity.

The relationship between breastfeeding and mean levels of adiposity levels in multivariable linear regression models is presented in Table 3. The effect of

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**Table 1. Characteristics of Study Participants**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current age, mean (SD), y</td>
<td>10.6 (1.3)</td>
</tr>
<tr>
<td>Male sex</td>
<td>216 (48.9)</td>
</tr>
<tr>
<td>Tanner stage &lt;2</td>
<td>227 (51.4)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>210 (47.5)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>191 (43.2)</td>
</tr>
<tr>
<td>African American</td>
<td>41 (9.3)</td>
</tr>
<tr>
<td>Total energy intake, mean (SD), kcal/d</td>
<td>1818.3 (559.6)</td>
</tr>
<tr>
<td>Energy from fat, mean (SD), % of total</td>
<td>35.65 (4.88)</td>
</tr>
<tr>
<td>Physical activity score, mean (SD)(^b)</td>
<td>82.80 (38.67)</td>
</tr>
<tr>
<td>Maternal educational level</td>
<td></td>
</tr>
<tr>
<td>&lt;High school</td>
<td>3 (0.7)</td>
</tr>
<tr>
<td>High school</td>
<td>56 (12.7)</td>
</tr>
<tr>
<td>Any college</td>
<td>383 (86.7)</td>
</tr>
<tr>
<td>Total household annual income</td>
<td></td>
</tr>
<tr>
<td>&lt;$50,000/y</td>
<td>94 (21.3)</td>
</tr>
<tr>
<td>$50,000/y</td>
<td>348 (78.7)</td>
</tr>
<tr>
<td>Maternal prepregnancy BMI, mean (SD) (^c)</td>
<td>25.6 (6.3)</td>
</tr>
<tr>
<td>Maternal smoking during pregnancy</td>
<td>27 (6.1)</td>
</tr>
<tr>
<td>Any breastfeeding</td>
<td>387 (87.6)</td>
</tr>
<tr>
<td>Any formula feeding</td>
<td>322 (72.9)</td>
</tr>
<tr>
<td>Average duration of breastfeeding, mo (^d)</td>
<td>7.6 (7.3)</td>
</tr>
<tr>
<td>Breast milk–months (^d)</td>
<td></td>
</tr>
<tr>
<td>Low, &lt;6 mo</td>
<td>230 (52.0)</td>
</tr>
<tr>
<td>Adequate, ≥6 mo</td>
<td>212 (48.0)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared). \(\*\)Study includes 442 children and adolescents. Unless otherwise indicated, data are expressed as number (percentage) of participants. \(\*\)Percentage score reflects the extent to which the number of 30-minute blocks of moderate-to-vigorous physical activity reported during the previous 3 days meets recommended levels. \(\*\)Includes mixed breastfeeding and formula feeding. \(\*\)Indicates weighted months of mixed feeding with breast milk and formula, calculated as follows: duration of exclusive breastfeeding (in months) + [duration of mixed breastfeeding and formula feeding (in months) \times exclusivity weight].

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**Table 2. Intercorrelations Adiposity Variables**

<table>
<thead>
<tr>
<th>Adiposity Variables</th>
<th>BMI</th>
<th>SAT</th>
<th>VAT</th>
<th>STR</th>
<th>IMCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT</td>
<td>0.93</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAT</td>
<td>0.67</td>
<td>0.71</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STR</td>
<td>0.51</td>
<td>0.53</td>
<td>0.47</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>IMCL</td>
<td>0.47</td>
<td>0.47</td>
<td>0.40</td>
<td>0.26</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; IMCL, intramyocellular lipid accumulation; SAT, subcutaneous adipose tissue deposition; STR, ratio of subcutaneous to triceps skinfold; VAT, visceral adipose tissue deposition. \(^a\)For all comparisons, \(P < .001\).
adequate vs low breastfeeding on mean levels of BMI, SAT, VAT, and STR tended to be protective but failed to reach statistical significance. Breastfeeding was not associated with IMCL levels in linear regression models (P = .84).

Results from our multivariable quantile regression analyses are presented in Table 4 and displayed graphically in the Figure. Adequate levels of breastfeeding were associated with a protective effect on adolescent BMI at the 85th and 95th percentiles, with an estimated reduction of −2.74 (P = .01) and −3.34 (P < .001), respectively, compared with youth with low breastfeeding levels. At the 30th percentile and below, adequate breastfeeding was associated with increased adolescent BMI, although the differences were not significant. At median BMI levels, the effect of adequate breastfeeding was null (−0.04 [P = .91]). A similar pattern was seen for SAT and STR, with adequate breastfeeding levels associated with lower SAT (−74.4 cm² [P = .02]) and STR (−0.19 [P = .01]) at the 95th percentiles and null effects at the 50th percentile (−1.47 cm² [P = .86] and −0.03 [P = .45], respectively). Statistically significant reductions in VAT levels associated with adequate neonatal breastfeeding were seen at somewhat lower percentiles, with −4.66 cm² (P < .001) at the 60th percentile, −4.70 cm² (P = .002) at the 70th percentile, and −5.20 cm² (P = .10) at the 95th percentile. No association between breastfeeding status and IMCL was noted with quantile regression.

This study provides evidence that breastfeeding reduces body size, VAT, and SAT and protects against central fat patterning among youth who are at upper percentiles of adiposity variables. Breastfeeding tends to increase BMI, SAT, VAT, and STR at lower percentiles and has null effects at the 50th percentile. Therefore, breastfeeding reduces variability in adiposity measures within a healthy, multiethnic population of children. Our findings support the notion that human breast milk is a unique and optimal nutrition for human growth and development, preventing extreme levels of body size and adiposity in either direction and shifting values to the mean.

The comparison of findings from our linear regression models and quantile analysis highlights the problem of assessing the impact of breastfeeding on mean levels of adiposity variables, whereas true shifts happen only at the tails of the distributions and potentially occur in opposite directions. Our analysis was based on an approach recently published by Beyerlein et al10 that assessed the impact of breastfeeding (any vs never) on BMI of 14,412 children aged 4.5 to 7.3 years in Bavaria, Southern Germany. The authors found no association by linear regression; however, by using quantile regression, they detected a reduction of BMI levels among children at the 90th percentile (−0.23 BMI units lower; 95% CI, −0.39 to −0.07) and 97th percentile (−0.26 BMI units lower; 95% CI, −0.45 to −0.07) associated with breastfeeding vs formula feeding. The authors found a null effect in the 40th through the 80th percentiles and higher BMI values below the 30th percentile. Our study observed a similar pattern in a much smaller population of 442 children aged 6 to 13 years from a diverse population of youth from Colorado. We assessed the impact of breastfeeding on overall body size and on measures of abdominal fat including VAT and SAT, indicators of central body fat.
distribution such as the STR, and IMCL levels. Our findings support those of the German study and add the observation that breastfeeding also prevents extreme levels of SAT, VAT, and centralized body fat distribution.

The potential protective effect of breastfeeding against obesity in childhood is a subject of much debate. Rapid infant growth has a clear relationship with obesity and cardiovascular disease, and formula feeding vs breastfeeding is associated with a more rapid weight gain in early infancy with an increased risk for later obesity. However, it has been argued that traditional analytic approaches do not adequately address the tempo-

Figure. Point estimates and 95% CIs (shaded areas) for differences in adiposity levels at selected percentiles between children with adequate and low levels of breastfeeding. The model was adjusted for age, sex, race/ethnicity, Tanner stage, Tanner × age interaction, current total daily caloric intake, current physical activity score, maternal smoking during pregnancy, level of education, income, and prepregnancy body mass index (BMI) (calculated as weight in kilograms divided by height in meters squared). A, Body mass index. B, Subcutaneous adipose tissue deposition (SAT). C, Visceral adipose tissue deposition (VAT). D, Ratio of subcutaneous to triceps skinfold (STR). E, Intramyocellular lipid accumulation (IMCL).
eral relationships between infant feeding practices and infant growth. Infants with slow initial weight gain may be more likely to receive formula supplementation or to be shifted entirely to formula feeding because of a fear of undernutrition. A recent analysis by Kramer et al30 based on the Promotion of Breastfeeding Intervention Trial (PROBIT) cohort found that smaller infants (with a weight-for-age z score of ≤1) had 20% to 60% higher odds of being weaned or discontinued from exclusive breastfeeding at 2 to 6 months of age than those with a weight-for-age z score greater than 1. In addition, faster-growing infants may cry more frequently owing to greater hunger demand, prompting mothers and/or their health care providers to conclude that the breast milk supply is not adequate and therefore to shift to supplementation.5 Quantile regression estimates are not dependent on the proportion of individuals who are breastfed in the upper and lower percentiles; thus, our findings do not reflect the fact that children in the upper percentiles of adiposity levels were more likely to be adequately breastfed. Rather, our results demonstrate that the effect of breastfeeding is differential across percentiles of adiposity outcomes, preventing extremes of body size, fatness, and deleterious fat accumulation.

Ectopic fat deposition in the soleus muscle (IMCL) was previously shown to be associated with abdominal and visceral fat and inversely associated with insulin sensitivity in adults and adolescents.29,30 We did not observe a relationship between breastfeeding and IMCL at any percentile distribution. We are not aware of other studies that have assessed the effect of breastfeeding on IMCL levels; however, our results suggest no relationship of breastfeeding with a marker of peripheral insulin sensitivity in this population of healthy young adolescents despite the observed reductions in VAT and abdominal obesity in the highest percentiles.

There are several potential limitations to our study. As is often the case with observational studies, we cannot completely rule out the possibility of residual confounding of our observed associations by socioeconomic status, current childhood lifestyle factors, and genetic predisposition, although our model included surrogate markers to adjust for these factors. Because our population was highly educated (>99% of mothers reported at least a high school education), our findings may not be entirely generalizable for populations with lower socioeconomic status. Our assessment of neonatal breastfeeding status was based on maternal self-report of events 6 to 13 years prior; however, maternal recall of breastfeeding after periods spanning 9 to 20 years has been found to correlate well with infant feeding data obtained from medical records (r = 0.86)31 or collected prospectively (r = 0.95).32 Our cohort of children and young adolescents, of whom 51.4% were prepubertal, are in a dynamic phase of change in fat deposition patterns, and this may have resulted in smaller or more variable differences in adiposity levels, such as visceral fat, by breastfeeding status.33 In addition, the relatively small cohort size (n = 442) may have increased the standard error at the tails of our distributions, thus limiting our ability to detect significant differences by breastfeeding status, especially at the lower end. Finally, the possibility of reverse causation exists, although the specific pattern of our results suggests a mechanistic relationship between breastfeeding and regulation of extremes of adiposity distribution in childhood. Our study also has important strengths. Our adiposity outcomes included direct, state-of-the-art measures of abdominal and peripheral adiposity and body fat distribution rather than relying solely on BMI, which may not adequately reflect overweight status in children and adolescents.34 Our cohort was racially and ethnically diverse and included non-Hispanic white, Hispanic, and African American participants. Finally, our measure of breastfeeding was based on a score that incorporates exclusivity and duration, although similar results were obtained when we considered breastfeeding as a yes/no variable or used simply breastfeeding duration.

In conclusion, this study provides evidence that breastfeeding selectively improves the extremes of childhood body size, abdominal fat deposition, and fat patterning. These data support the notion that early postnatal life has long-term influences on growth and obesity-related disease risk and suggests that obesity prevention should start in the perinatal period.

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Author Contributions: Dr Crume had access to all the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis. Study concept and design: Crume and Dabelea. Acquisition of data: Crume, Scherzinger, Stamm, and Dabelea. Analysis and interpretation of data: Crume, Bahr, Mayer-Davis, Hamman, and Dabelea. Drafting of the manuscript: Crume. Critical revision of the manuscript for important intellectual content: Crume, Bahr, Mayer-Davis, Hamman, Scherzinger, Stamm, and Dabelea. Statistical analysis: Crume, Bahr, Mayer-Davis, and Dabelea. Obtained funding: Dabelea. Administrative, technical, and material support: Crume, Scherzinger, and Stamm. Study supervision: Scherzinger and Dabelea.

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