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Body mass index, adiposity rebound and early feeding in a longitudinal cohort (Raine Study)

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Objective: This study examined the influence of type and duration of infant feeding on adiposity rebound and the tracking of body mass index (BMI) from birth to 14 years of age.

Methods: A sample of 1330 individuals over eight follow-ups was drawn from the Western Australian Pregnancy Cohort (Raine) Study. Trajectories of BMI from birth to adolescence using linear mixed model analysis investigated the influence of age at which breastfeeding was stopped and the age at which other milk was introduced (binomial 4-month cutoff point). A subsample of linear mixed model-predicted BMI was used to determine BMI and age at nadir for early infant feeding groups.

Results: Chi-square analysis between early feeding and weight status (normal weight, overweight and obese) groups found a significant difference between the age at which breastfeeding was stopped ($P<0.001$) and the age at which other milk was introduced ($P=0.011$), with a higher proportion of overweight and obese in the ≤4-month group, even after controlling for maternal education. Using the linear mixed model, the BMI determined was higher over time for the group that was breastfed for ≤4 months ($P=0.015$), with a significant interaction effect with the group in which other milk was introduced at ≤4 months ($P=0.011$). Using predicted BMI from the linear mixed model, significant differences for nadirs of adiposity rebound between early feeding groups were found ($P<0.005$).

Conclusions: Early infant feeding was important in the timing of, and BMI at, adiposity rebound. The relationship between infant feeding and BMI remained up to the age of 14 years. Although confounding factors cannot be excluded, these findings support the importance of exclusive breastfeeding for longer than 4 months as a protective behaviour against the development of adolescent obesity.

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Keywords: adiposity rebound; body mass index; breastfeeding; child; linear mixed models; Raine study

Introduction

The type and duration of infant feeding may have an important role in the development of biological and behavioural processes, affecting subsequent growth and health.¹,² Debate continues as to whether breastfeeding is protective against, or predictive of, childhood obesity, or rather uncontrolled bias by other confounders.³–⁷ Most likely, biological, hormonal and behavioural mechanisms are implicated.⁷ Recent reviews and meta-analyses suggest that longer duration of exclusive breastfeeding may be protective against later obesity.⁶,⁷ Increasingly, breast and formula feeding are being co-investigated, particularly in relation to later weight status.⁸–¹¹

The impact of breastfeeding on the timing of adiposity rebound requires clarification.²,¹² In adiposity rebound, the body mass index (BMI) curve increases during the first year of life so that 1-year-old children seem chubby, but the curve decreases after the first year to about 6 years of age when fatness increases again. The duration of fatness decrease after 1 year of age varies between children so that adiposity rebound can occur between 4 and 8 years of age, with the
earlier the rebound the higher the adiposity at the end of growth. Among children who become obese, adiposity rebound occurs as early as 3 years of age, compared with about 6 years of age for children of normal BMI.13

Adipocyte cell size increases during the first year of life and then decreases, increasing again from ~6 years of age. Transient obesity in early childhood could involve the increase in cell size, but persistent obesity commencing with an early adiposity rebound could be associated with early cell multiplication.2 For this reason, an understanding of the role of infant feeding mode on the timing of adiposity rebound is of utmost importance.

This study examines the influence of early feeding (age at which breastfeeding stopped and age at which other milk was introduced) on later weight status, controlling for gender and gestational age, using linear mixed model BMI trajectories to 14 years in a longitudinal pregnancy cohort.14 Infant feeding measures are investigated with respect to adiposity rebound and weight status at 14 years. Demonstration of a relationship between early feeding and the timing of, and BMI at, adiposity rebound may provide further evidence of early infant feeding being an early preventive mechanism against the development of later obesity.

Methods

The Western Australian Pregnancy Cohort (Raine) Study has been previously detailed with 2868 children followed up from birth, with data collected at follow-ups 1, 2, 3, 6, 8, 10 and 14 years of age. The protocol for the original study has been reported for antenatal15 and postnatal8 periods.

Data on multiple birth, congenital abnormality and preterm birth (gestational age <37 weeks) cases were excluded from the original sample (n = 369). Only participants with a recorded gestational age and BMI at year 14 were included (male = 729, female = 674). Early feeding behaviours were age at which breastfeeding was stopped (n = 1330) and age at which other milk was introduced (n = 1320), with details provided in Table 1.

Anthropometric measures

Standard anthropometric assessments of height and weight, using strict protocols, were conducted by a small group of extensively trained staff.14 Length was measured to the nearest 0.1 cm using the Harpenden Neonatometer (Holtain Ltd., Crosswell, UK) at birth and at 1-year follow-up, and thereafter using a Holtain stadiometer (Holtain Ltd.). Weight was assessed to the nearest 100 g, using calibrated hospital scales at birth and Wedderburn digital chair scales (Wedderburn, Australia) thereafter.

Early infant feeding

Parent recall to specific questions in follow-ups conducted at 1, 2 and 3 years collected information pertaining to early infant feeding. Mothers reported the age at which breastfeeding was stopped (in months) and the age at which milk other than breast milk was introduced (in months). Milk other than breast milk typically included infant formula (67.7%), cow (87.7%) or soy milk (20.8%). Age at which breastfeeding was stopped and the age at which milk other than breast milk was introduced were determined from the

Table 1 Summary of sample statistics, separated for males and females

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>729 (52)</td>
<td>674 (48)</td>
<td>1403</td>
</tr>
<tr>
<td>Frequency of normal weight (%)</td>
<td>530 (72.7)</td>
<td>501 (74.3)</td>
<td>1031 (73.5)</td>
</tr>
<tr>
<td>Frequency of overweight (%)</td>
<td>136 (18.7)</td>
<td>127 (18.8)</td>
<td>263 (18.7)</td>
</tr>
<tr>
<td>Frequency of obese (%)</td>
<td>63 (8.6)</td>
<td>46 (6.8)</td>
<td>109 (7.8)</td>
</tr>
<tr>
<td>Mother’s education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No post-secondary (%)</td>
<td>333 (45.7)</td>
<td>321 (47.6)</td>
<td>654 (46.6)</td>
</tr>
<tr>
<td>Non-professional post-secondary (%)</td>
<td>172 (23.6)</td>
<td>164 (24.3)</td>
<td>336 (23.9)</td>
</tr>
<tr>
<td>Professional tertiary (%)</td>
<td>224 (30.7)</td>
<td>189 (28.0)</td>
<td>413 (29.4)</td>
</tr>
<tr>
<td>N (%)</td>
<td>690 (52)</td>
<td>640 (48)</td>
<td>1330</td>
</tr>
<tr>
<td>Mean age at which breastfeeding was stopped (in months (s.d.))</td>
<td>8.0 (6.8)</td>
<td>7.8 (7.0)</td>
<td>7.9 (6.9)</td>
</tr>
<tr>
<td>Two-category breastfeeding, frequency (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤4 months</td>
<td>268 (38.8)</td>
<td>253 (39.5)</td>
<td>521 (39.2)</td>
</tr>
<tr>
<td>&gt;4 months</td>
<td>422 (61.2)</td>
<td>387 (60.5)</td>
<td>809 (60.8)</td>
</tr>
<tr>
<td>N (%)</td>
<td>686 (52)</td>
<td>634 (48)</td>
<td>1320</td>
</tr>
<tr>
<td>Mean age at which other milk was introduced (in months (s.d.))</td>
<td>5.1 (4.1)</td>
<td>5.0 (4.1)</td>
<td>5.0 (4.1)</td>
</tr>
<tr>
<td>Two-category other milk introduced, frequency (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤4 months</td>
<td>356 (51.9)</td>
<td>332 (52.4)</td>
<td>688 (52.1)</td>
</tr>
<tr>
<td>&gt;4 months</td>
<td>330 (48.1)</td>
<td>302 (47.6)</td>
<td>632 (47.9)</td>
</tr>
</tbody>
</table>

*Weight status groups determined at 14 years of age using International Obesity Task Force criteria.
mother’s diary of early feeding milestones, as well as from an interview with the study nurse and survey questions at later follow-ups. Similar to Burke et al., who also analysed data for this cohort, no distinction between exclusive or partial breastfeeding was made.

Statistical processes
Statistical analyses were undertaken using SPSS version 17 (SPSS Inc, Chicago, IL, USA). In each follow-up, BMI was calculated from measured height and weight scores using the formula weight in (kg) per height in (m)². At birth and at year 1, a proxy for BMI was used (weight per length²). BMI cutoff points equivalent to 25 and 30 kg m⁻², adjusted for age and gender, classified the participants as normal weight, overweight or obese, as defined by the International Obesity Task Force criteria, at 14 years. For males at 14 years, the cutoff point is 22.62 and 27.63 kg m⁻², whereas for females, it is 23.34 and 28.57 kg m⁻², for overweight and obese, respectively.

Adiposity rebound, defined as the last minimum (nadir) BMI before the continuous increase with age, was calculated in a subset of individuals (n = 171) for whom a complete set of BMI data were available. This small sample was because of limited anthropometric measurements taken at the 2-year follow-up (insufficient funding), but is similar in size to another study. Adiposity rebound was based on the child’s age in months. Given the wide range of participant ages in the 3- and 6-year follow-ups, data are available for 3–4 years, and for 5.5 to 6 years, leaving a gap between 4 and 5.5 years without data. This is pertinent to the calculation of adiposity rebound. BMI and age at nadir were calculated for both raw BMI and predicted BMI (based on the longitudinal linear mixed model that adjusts for age, gender, weight status and gestational age).

The variables for age at which breastfeeding was stopped and age at which other milk was introduced were categorized using a 4-month cutoff point (categories ≤ 4 months and > 4 months). This cutoff point splits the cohort almost equally, and was the World Health Organization recommendation for the duration of exclusive breastfeeding at the time these data were collected (1990–1993). We investigated these covariates individually and together in the linear mixed model.

Independent t-tests were used to examine early feeding group differences for BMI and age at nadir and gender differences for the mean age at which breastfeeding was stopped and the age at which other milk was introduced. Analysis of variance (ANOVA) and post hoc tests determined the differences between weight status groups. Binomial variables (based on the 4-month cutoff point) were compared across weight status groups at 14 years, and group differences were tested using the Pearson χ²-test. Logistic regression (ordinal and multinomial) adjustment for potential socioeconomic status confounding was based on maternal education, family income, maternal pre-pregnancy weight status and father’s occupation.

The influence of early feeding on weight status at 14 years was analysed using a previously reported model of BMI trajectories, developed using a linear mixed model. The original linear mixed model investigated fixed and random effects, interactions, covariance structure and nonlinear transformations of age, with no replacement of missing values. Time was used as a repeated measure. In the final linear mixed model, BMI was treated as the dependent variable, with gender, age, age squared, natural log of age, gestational age and weight status treated as factors (fixed effects). In this study, the age at which breastfeeding was stopped and the age at which other milk was introduced were added as fixed effects.

Results
There was a slight selection bias in our study, similar to the retention trends seen across the larger databases from each follow-up in the Raine Study. The early feeding sample tended to have a higher proportion of professional fathers (21.8% vs 14.7%) and high-income families (36.6% vs 24.9%), with a lower proportion of mothers aged < 20 years (7.3% vs 13.7%), compared with the sample not selected, with no statistical differences in gestational age, gender or family structure.

Previously shown in this cohort, and similar to other studies, mothers with a low income and a higher maternal BMI were more likely to formula feed. There was a gender difference in birth weight, with males being heavier than females (P < 0.001). Overall, there were no statistical differences between birth weight and weight status, although when analysed separately for gender, a significant difference was found between normal weight and overweight females only (P = 0.04).

There was a gender difference in maturation, as determined by the development of pubic hair (Tanner stages)²⁶, with a greater proportion of girls in the later stages of puberty, compared with boys (P < 0.001). There were no statistical differences between weight status groups and pubertal stages for either boys or girls; therefore, maturation was not considered as a confounder in this study.

No significant gender differences for age at which breastfeeding was stopped or age at which other milk was introduced were observed (Table 1). Comparison of the two-group category, namely, age at which breastfeeding was stopped and age at which other milk was introduced, using the 4-month cutoff point and weight status at the age of 14 years are reported in Table 2. The χ²-analysis found a significant difference between the age at which breastfeeding was stopped (P < 0.001) and the age at which other milk was introduced (P = 0.011) with weight status groups at 14 years. The groups that included those who had been breastfed for ≤ 4 months or were introduced to other milk at ≤ 4 months contained a significantly higher proportion of overweight
BMI modelling

The influence of early feeding on BMI over time was investigated by adding the early feeding covariates to a previously reported linear mixed model. The best model, based on an assessment of Akaike's information criterion, included the combined categorical (4-month cutoff) model, with age at which breastfeeding was stopped and age at which other milk was introduced as fixed effects, and an interaction effect between them. Fixed effects from the original model included weight status calculated at 14 years, gender, age, age squared, log(age), gestational age with interactions between gender and age variables, and weight status and age variables.

Body mass index was consistently higher over time for the group breastfed for \( \leq 4 \) months \((P = 0.015)\) and for those introduced to other milk at \( \leq 4 \) months, although it was not significant \((P = 0.105)\) (Figure 1). Analysis of linear mixed model estimates showed that individuals who were breastfed for \( \leq 4 \) months and started on other milk at \( \leq 4 \) months showed a decreased BMI over time \((P = 0.011)\); introducing other milk before 4 months, but breastfeeding beyond 4 months (mixed feeding) resulted in the largest increase in BMI over time, when compared with the groups who were breastfed and introduced to other milk after 4 months. These differences remained after adjusting for maternal education, family income, maternal pre-pregnancy weight status and

| Table 2 | Summary statistics for early infant feeding groups and BMI weight status groups at 14-years of age |
|-----------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| BMI weight status determined at age 14 years | Total | Normal weight | Overweight | Obese | \( \chi^2 \) values | P-values |
| Age at which breastfeeding was stopped | 1330 | 977 | 248 | 105 | 15.218 | <0.001 |
| \( \leq 4 \) months | 521 | 353 (67.8%) | 114 (21.9%) | 54 (10.4%) | 4.721 | 0.096 |
| >4 months | 809 | 624 (77.1%) | 134 (16.6%) | 51 (6.3%) | 6.051 | 0.048 |
| Age at which other milk was introduced | 1320 | 969 | 246 | 105 | 9.062 | 0.011 |
| \( \leq 4 \) months | 688 | 481 (69.9%) | 144 (20.9%) | 63 (9.2%) | 7.071 | 0.029 |
| >4 months | 632 | 488 (77.2%) | 102 (16.2%) | 42 (6.6%) | 5.038 | 0.024 |

Abbreviation: BMI, body mass index.

and obese adolescents (32.3 and 30.1%, respectively) compared with those who were breastfed longer or were started on other milk later (22.9 and 22.8%, respectively).

Adjustment results for socioeconomic confounders are reported in Table 3. The age at which breastfeeding was stopped remained significantly associated with later obesity when adjusted for maternal education, income and mother’s pre-pregnancy weight status. The association was not significant when adjusted for father’s occupation, nor when all confounders were included in the model. The age at which other milk was introduced (introduced) remained significantly associated with later obesity when adjusted for maternal education and income. However, the association was not significant when adjusted for maternal pre-pregnancy weight status and father’s occupation, nor when all confounders were included in the model. There were no significant modification effects for gender.
father's occupation. When all covariates were modelled together, only maternal pre-pregnancy weight status remained significant. For this model, BMI of children with a normal weight mother decreased by 0.43 kg m\(^{-2}\) (\(P < 0.001\)), that of children with an overweight mother decreased by 0.23 kg m\(^{-2}\) (\(P = 0.082\)), stopping breastfeeding before the age of 4 months increased BMI by 1.19 kg m\(^{-2}\) (\(P = 0.026\)) and starting other milk before the age of 4 months increased BMI by 0.134 kg m\(^{-2}\) (\(P = 0.096\)). Overall, starting other milk before the age of 4 months, but breastfeeding beyond 4 months, reported the highest increases in BMI.

**Adiposity rebound**

Significant differences between weight status groups for BMI and age at nadir were identified in a subset of 171 individuals (\(P < 0.010\)), with similar results for males and females. Adiposity rebound occurred for the normal weight group at 5.3 years (s.d. = 2.2), for the overweight group at 3.8 years (s.d. = 2.2) and for the obese group at 2.6 years (s.d. = 1.4). There were no statistically significant differences between the overweight and obese groups in the timing or BMI at adiposity rebound.

There were no significant differences between the age at which breastfeeding was stopped and the age at which other milk was introduced for raw BMI and age at nadir (Figure 1). However, using predicted BMI based on the linear mixed model, adjusted for age, gestational age, gender and weight status, significant differences in both BMI and age at nadir were found (Table 4). The timing of adiposity rebound (age at nadir) was earlier (\(P = 0.005\)) and the BMI at nadir was higher (\(P = 0.003\)) for the group that stopped breastfeeding at \(\leq 4\) months. Only BMI at nadir was higher (\(P = 0.002\)) for the group that was introduced to other milk at \(\leq 4\) months.

**Discussion**

This study found that early infant feeding influenced the timing and BMI at adiposity rebound and that this influence remained until adolescence, which to our knowledge has not previously been reported. The age at which breastfeeding was stopped and the age at which other milk was introduced had a significant role in the trajectory of BMI from birth to 14 years, especially in the 4-month cutoff-point group.
differences for BMI peak at age 1 year, with this difference remaining consistent over time (Figure 1). Importantly, the modelling process indicated that the age at which breastfeeding was stopped and the age at which other milk was introduced should be investigated simultaneously. Interestingly, as indicated by the linear mixed model results, those individuals who were breastfed for > 4 months but were introduced to other milk at \( \leq 4 \) months (mixed feeding) had the highest increase in BMI. This finding supports ‘reverse causality’, in which a mother’s perception of infant hunger or need for enhanced growth may influence her decision to supplement with other milk and may result in overfeeding. On the other hand, those individuals who were breastfed for > 4 months and were started on other milk at > 4 months did not increase their BMI. In short, formula feeding may reduce self-regulation, with parental behaviour overriding satiety, whereas breastfeeding provides protection against overfeeding. These results support other studies that found that overweight in adolescence decreased as time spent being exclusively breastfed increased. Given that many unmeasured factors may affect a mother’s early feeding choice, these differences in exclusive and mixed feeding behaviours may be precursors for later dietary behaviours, and may explain why tracking over time is seen in this cohort.

Similar to Blair et al., for their New Zealand cohort, we concur that preschool years are a critical time period for the development of obesity, and that early feeding has an important role, possibly in the establishment of dietary behaviours. Our results extend the findings of Burke et al. with this cohort that showed a higher BMI at age 8 years in children breastfed for less than 4 months. A cross-sectional analysis of early infant feeding and weight status at 14 years showed that the proportion of overweight and obese was higher in those who experienced mixed feeding. We observed a difference in BMI peak at the age of 1 year for the different weight status groups, supporting the theory that early introduction of formula feeding is related to growth acceleration and to overweight or obesity, whereas exclusive breastfeeding is more likely to meet the nutrient and energy requirements of each child. Our finding that BMI differences between early infant feeding groups is still evident at age of 14 years may indicate that breastfeeding develops behavioural mechanisms for food acceptance and control of energy intake, but whether this is self-regulated or parent regulated by unmeasured confounders is unknown.

The timing of adiposity rebound may be identified as a marker of later obesity, with our data depicting distinct and significantly different pathways for the three weight status groups. As shown in Figure 1, those who were breastfed for > 4 months had a lower mean BMI. Failure to detect a statistical difference between raw BMI and adiposity rebound measures may be related to the small sample size available for analysis using traditional methods. The linear mixed model predicted BMI on the basis of the whole sample while accounting for age, gestational age, gender and weight status. Using our data to statistically model population behaviour, breastfeeding was shown to have an important role in the timing of adiposity rebound, and both breastfeeding and the age at which other milk was introduced were important contributors to BMI at adiposity rebound. Our results support early feeding literature that suggests that bottle feeding compared with breastfeeding results in accelerated weight gain in the infant with probably upward BMI centile crossing, as depicted in the adiposity rebound nadir results.

**Strengths and limitations**

The sample described was not drawn randomly, but enrolled in utero from a major women’s hospital in Perth, Western Australia, and therefore the findings may not be truly representative. There was an expected attrition rate, with variation across follow-ups and among measured variables, although overall participant numbers remained high.

With respect to adiposity rebound, a major limitation was the absence of data collected between the ages of 4 and 5 years, although Rolland-Cachera et al. report adiposity rebound results using 0.8, 2, 4, 6 and 8 years. The absence of data could account for a lack of detection of a statistical difference between the overweight and obese weight status groups. However, we found a statistical difference in BMI at nadir, with the normal weight group having a lower BMI at rebound compared with the overweight and obese groups.

The modelled BMI based on predicted population behaviour showed important differences between weight status groups.

Another limitation was the inability to accurately discern mixed feeding practices and investigate this group cross-sectionally. In particular, no information was gathered on the duration of mixed feeding or on the proportions of breast milk and other milk that were being fed. The linear mixed model showed that the group that breastfed for more than 4 months and that was supplemented with other milk before 4 months was the most at risk group for increased BMI. It is possible that mixed feeding has an important association with later obesity.

This study extends the findings previously reported by Burke et al. on breastfeeding and adiposity, providing two additional follow-ups at 10 and 14 years. The strength of our study was the unique linear mixed model that accounted for correlated errors normally associated with repeated, continuous and correlated observations, providing an opportunity to examine early pathways of weight status, in particular, the possible influence of early feeding on adolescent BMI and its relationship with adiposity rebound.

Recent reports have thrown into question whether the association between breastfeeding and subsequent obesity is causal or in fact an uncontrolled bias, mediated by selection and confounding socioeconomic environmental issues that determine parental family feeding and physical activity. Reviews of breastfeeding and adiposity studies found that breastfeeding is protective against later obesity, although...
when potential confounders are considered, there is an attenuation of risk.\textsuperscript{5} Maternal education,\textsuperscript{26} maternal socioeconomic status, low birth weight,\textsuperscript{5} maternal obesity\textsuperscript{2,19,23} and maternal diabetes\textsuperscript{5,23} are all potential confounders, along with unmeasured sociocultural factors.\textsuperscript{31} In this study, preterm and multiple births were excluded and adjustment was made for gestational age. Physical maturity was not a confounder. Adjustment for socioeconomic status variables such as maternal education, family income, maternal pre-pregnancy weight status and father’s occupation yielded mixed results in cross-sectional analysis using logistic regression; however, the linear mixed model showed that only maternal pre-pregnancy weight status remained significant, with the influence of early infant feeding remaining significant in the longitudinal model of BMI from birth to age 14 years.

**Conclusion**

Subject to reservations regarding possible unrecognized or unmeasured confounders, the age at which breastfeeding is stopped and the age at which other milk is introduced may be influential on later BMI, at least until the age of 14 years. The linear mixed model results also indicate that mixed feeding practices may account for the greatest increase in BMI. Investigation of adiposity rebound using the modelled BMI found statistical differences between groups in the age of nadir for age at which breastfeeding was stopped, and the BMI at nadir for both the age at which breastfeeding was stopped and the age at which other milk was introduced. This supports the concept of early infant feeding as an important contributor to BMI pathways from birth to later adolescence and an important mechanism for delaying adiposity rebound.

This study supports the importance of exclusive breastfeeding for more than 4 months as a protective factor against the development of later adolescent obesity, and highlights an integral role that the timing of the introduction of milk other than breast milk has on a child’s BMI later in life. Further research on mixed and exclusive feeding practices and their confounders, particularly maternal weight, may provide a better understanding of their role in developing later dietary patterns that may influence weight status in adolescents and adulthood.

**Conflict of interest**

The authors declare no conflict of interest.

**Acknowledgements**

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