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Infant diet and subcutaneous fat mass in early childhood: The Generation R Study

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Background/Objectives: Breastfeeding has a protective effect on childhood obesity, but the influences on body composition in early childhood are not known. The objective of this study is to assess whether the duration and exclusiveness of breastfeeding, and the timing of introduction of solid foods are associated with the subcutaneous fat mass in early childhood.

Subjects/Methods: This study was embedded in a population-based prospective cohort study among 779 children. Peripheral (biceps, triceps) and central (suprailiacal and subscapular) subcutaneous fat mass was measured as skinfold thickness at the ages of 1.5, 6 and 24 months.

Results: Breastfeeding duration was not associated with subcutaneous fat mass at the age of 1.5 months. Shorter breastfeeding was associated with higher peripheral and total subcutaneous fat mass at the age of 6 months (P -value for trend <0.05), but not at the age of 24 months. As compared to children who were exclusively breast fed for 4 months, those who were non-exclusively breast fed had a higher central fat mass at the age of 24 months (P -value for trend <0.01). Timing of introduction of solid foods was not associated with subcutaneous fat mass.

Conclusion: Our results suggest that a shorter duration and non-exclusive breastfeeding affect early body composition during the first 2 years of life. Follow-up studies at older ages are needed to explore the long-term consequences.

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Keywords: infant feeding; obesity; breastfeeding; solid foods; skinfold; pediatrics

Introduction

Several studies showed a protective effect of breastfeeding on the risk of overweight in children and adults (Arenz *et al.*, 2004; Harder *et al.*, 2005; Owen *et al.*, 2005a,b). Although the effect estimates for the associations of breastfeeding with mean body mass index are generally small, they seem to be consistent (Bergmann *et al.*, 2003; Arenz *et al.*, 2004; Harder *et al.*, 2005; Owen *et al.*, 2005a,b). An inverse dose-dependent association has been shown, suggesting that longer duration of breastfeeding is associated with a lower

body mass index (Arenz *et al.*, 2004; Harder *et al.*, 2005). Some studies have also suggested that early weaning may increase body mass index in childhood, but results are inconsistent (Forsyth *et al.*, 1993; Wilson *et al.*, 1998; Morgan *et al.*, 2004; Wright *et al.*, 2004). Body mass index provides only information about body weight, whereas it does not distinguish between fat and lean mass (Nevill *et al.*, 2006; Wells and Fewtrell, 2006). Because an unfavorable fat distribution may be stronger related to cardiovascular and metabolic diseases, it is important to explore the associations of breastfeeding with measures of fat distribution. Only a few studies have examined the relationships between breastfeeding in infancy and direct measures of adiposity in childhood, but no consistent associations were observed (Burdette *et al.*, 2006; Gale *et al.*, 2007). Skinfold thickness (SFT) is a valid measurement for subcutaneous fat mass assessment in epidemiological studies (Nevill *et al.*, 2006; Wells and Fewtrell, 2006). These measurements are

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quick and simple to obtain in most age groups, including young infants (Wells and Fewtrell, 2006). We have previously shown that birth weight is associated with subcutaneous fat mass in early childhood (Ay *et al.*, 2009). Subcutaneous fat mass also tends to track throughout early childhood (Ay *et al.*, 2008).

We examined in a population-based prospective cohort study among 779 children, the associations of breastfeeding duration and exclusivity, and timing of introduction of solid foods with peripheral, central and total subcutaneous fat mass in early childhood.

Materials and methods

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children from fetal life onwards in Rotterdam, the Netherlands (Jaddoe *et al.*, 2010). Enrollment in this study was aimed at early pregnancy (gestational age <18 weeks) but was possible until birth of the child. All children were born between April 2002 and January 2006, and form a prenatally enrolled birth-cohort that is currently followed until young adulthood. Additional detailed assessments of the postnatal growth and development were conducted in a subgroup of 1106 Dutch mothers and their children from late pregnancy (Jaddoe *et al.*, 2010). Between February 2003 and April 2005, all pregnant mothers participating in the Generation R study, who met this criterion, were approached for additional measurements (Jaddoe *et al.*, 2010). Of all approached women, 80% agreed to participate in the subgroup study. The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam. Written informed consent was obtained from all parents.

Population for analysis

From the total of 1106 children, 1039 children participated in at least one of the postnatal assessments at the ages of 1.5, 6 and 24 months (Figure 1). Children without complete information on breastfeeding ($n=225$) were excluded from the analyses. Of the remaining 814 live births with complete data on breastfeeding, skinfold measurements were measured in at least one of the three visits in 799 children. Next, twins ($n=20$) were excluded from the analyses because twins are correlated and may differ from other children in the relation between breastfeeding and subcutaneous fat mass. Missing skinfold measurements were mainly due to crying behavior. No differences in child and maternal characteristics were found between children with and without skinfold measurements ($n=779$ versus 1106).

Duration and exclusiveness of breastfeeding and solids foods introduction

Information about breastfeeding initiation and continuation was obtained from delivery reports and postal questionnaires at the ages of 2, 6 and 12 months after birth, as described previously (Durmuş *et al.*, 2011). Mothers were asked whether they ever breast fed their child (yes; no) and at what age they quit breastfeeding. Subsequently, breastfeeding duration was categorized into three groups: (i) never; (ii) <4 months and (iii) 4 months or longer. Duration of exclusive breastfeeding was defined by using information about at what age other types of milk and/or solids were introduced in the first 6 months of life, according to a short food frequency questionnaire. The information about duration and exclusiveness of breastfeeding was combined and categorized into the following three categories: (i) never; (ii) non-exclusive breast fed until 4 months and (iii) exclusive breast fed until 4 months. Never indicates infants who were never breast fed. Non-exclusive indicates infants receiving both breastfeeding, and formula feeding or solids during the first 4 months. Exclusive indicates infants who have been breast fed, without any other milk, solids or fluids during the first 4 months. Information about timing of introduction of solid foods such as fruit- and vegetable snacks was obtained from the same short food frequency questionnaire. The starting age of solid foods was defined as the age at which a fruit- or vegetable snack was given for the first time (<4 months; 4–5 months; and >5 months).

Subcutaneous fat mass measurements and anthropometrics

Subcutaneous fat mass was measured as SFT in millimeters at the ages of 1.5, 6 and 24 months on the left side of the body at four different sites (biceps, triceps, suprailiacal and subscapular) according to the standard procedures by using a skinfold caliper (Slim Guide, Creative Health Products, USA) (Lohman *et al.*, 1991). Four well-trained medical assistants performed all measurements (WHO, 2006). The consensus between and among observers for the medical assistants was analyzed using the intraclass correlation coefficient (Shrout and Fleiss, 1979; Bland and Altman, 1986). Intraobserver intraclass correlation coefficient was 0.88 and interobserver intraclass correlation coefficient was 0.76. The total subcutaneous fat mass was calculated from the sum of biceps SFT, triceps SFT, suprailiacal SFT, subscapular SFT. Central subcutaneous fat mass was calculated from the sum of suprailiacal SFT, subscapular SFT. Peripheral subcutaneous fat mass was calculated from the sum of triceps SFT, biceps SFT (Birmingham *et al.*, 1993; Ketel *et al.*, 2007). Body length at the age of 1.5 and 6 months was measured in supine position to the nearest millimeter using a neonatometer and in 24-month olds height was measured in standing position by a Harpenden stadiometer (Holtain Limited, Dyfed, UK). Weight was measured to the nearest grams in naked infants at the age of 1.5 and 6 months by

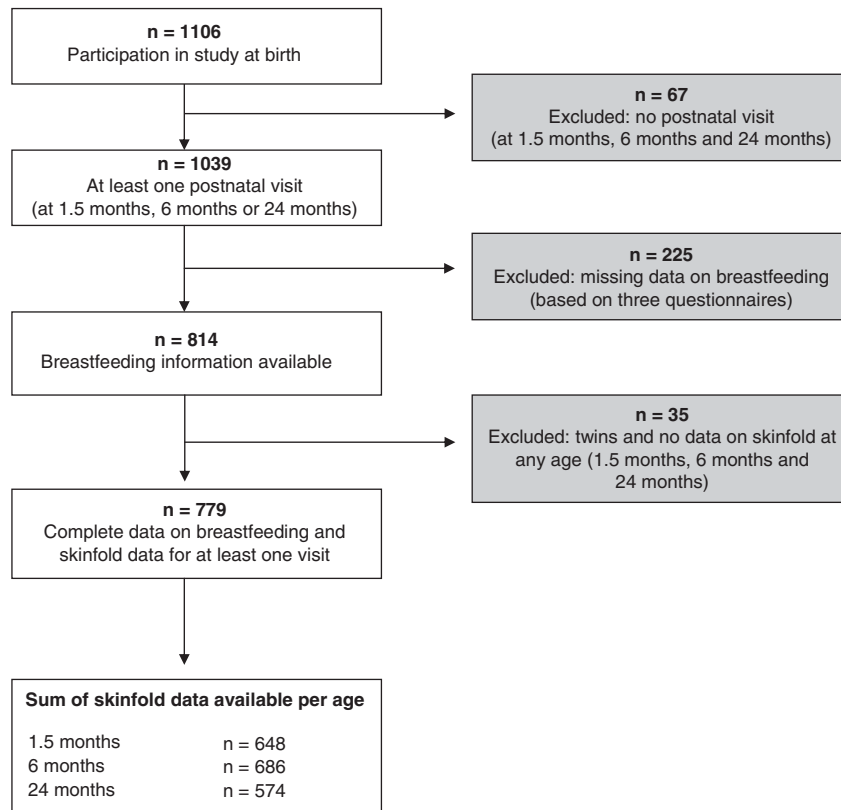


Figure 1 Flow chart of participants in this study.

using an electronic infant scale (SECA, Almere, The Netherlands) and in 24-month olds by a mechanical personal scale (SECA).

Covariates

Gestational age at birth, birth weight and sex were obtained from midwife and hospital registries at birth. Information about highest attained maternal educational level and parity were obtained at enrollment in the study. Educational level of the parents was defined according to the classification of Statistics Netherlands (Statistics Netherlands, 2004). Information on maternal smoking during pregnancy (yes; no) was retrieved from the prenatal questionnaire. Maternal height and weight were measured at enrollment although the mother stood without shoes and heavy clothing, and body mass index was calculated (kg/m^2).

Statistical analysis

Differences in baseline characteristics between the breastfeeding variables ever (never; ever), duration (<4 months; >4 months) and exclusivity (non-exclusive breast fed until 4 months; exclusively breastfed until 4 months) were compared with Student's *t*-test for continuous variables and χ^2 -tests for categorical variables. The associations of breast-

feeding (never; ever), breastfeeding duration (never; <4 months; and >4 months), breastfeeding exclusivity (never; non-exclusive until 4 months; and exclusive until 4 months) and timing of introduction of fruit-and vegetable snacks (<4 months; 4–5 months; and >5 months) with peripheral, central and total subcutaneous fat mass at the ages of 1.5, 6 and 24 months, were assessed using multiple linear regression models. The reference groups of these regression models were ever breast fed, breastfeeding duration of >4 months, exclusive breast fed until 4 months, respectively. In these models, breastfeeding categories were used as predictor variables and peripheral, central and total fat as outcome variables. The models were adjusted for potential confounders including child's age at visit, sex, maternal education, maternal body mass index, smoking and parity, gestational age, birth weight, current height and observer. The models focused on the role of timing of introduction of solid foods, were additionally adjusted for breastfeeding duration. Tests for trends were performed by treating each categorized variable as a continuous term and by entering the variable into the fully adjusted linear regression model. To handle missing values in covariates, we performed multiple imputations by generating five independent data sets for all analyses using the Markov Chain Monte Carlo method. Imputations were based on the relationships between all

covariates included in this study. All measures of association are presented with their 95% confidence intervals. Statistical analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Subject characteristics are given in Table 1. Of the total group of 779 children, 87.9% had ever been breastfed with a mean duration of 4.5 months (range 0.5–12.0). Differences between the breastfeeding categories are given in the Supplementary Material (Supplementary Tables S1–S3).

Table 1 Subject characteristics^a

	Total (n = 779)
<i>Maternal characteristics</i>	
Age (years)	32.3 (24.9–37.9)
Height (cm)	170.8 (6.3)
Weight (kg)	72.0 (13.4)
Body mass index (kg/m ²)	24.7 (4.4)
<i>Highest educational level (%)</i>	
Low	1.8
Moderate	34.5
High	63.7
<i>Smoking in pregnancy (%)</i>	
Ever	22.2
Never	77.8
<i>Alcohol consumption in pregnancy (%)</i>	
Ever	70.1
Never	29.9
<i>Parity (%)</i>	
0	62.5
≥1	37.5
<i>Birth characteristics</i>	
Males (%)	51
Gestational age (weeks)	40.3 (37.1–42.1)
Weight (g)	3515 (549)
Small for gestational age (<5%)	4.9
Low birth weight (<2500 g; %)	3.6
Preterm birth (%)	4.1
<i>Breastfeeding</i>	
Ever (%)	87.5
Duration (months)	4.5 (0.0–12.0)
<i>Solid foods (fruit or vegetables)</i>	
<4 months introduced (%)	7.7
>4–5 months introduced (%)	60.4
>5 months introduced (%)	31.9

^aValues are means (s.d.), percentages or medians (90% range) for variables with skewed distribution. Of the total group, data were missing on maternal anthropometrics (n = 5), maternal education (n = 9), maternal smoking (n = 98), maternal alcohol use (n = 93), parity (n = 1), breastfeeding duration (n = 141) and introduction solid foods (n = 153).

As compared with mothers who never breast fed their children, those who breast fed their children had more frequently a higher educational level, were more likely to consume alcohol during pregnancy but less likely to smoke (Supplementary Table S1). Mothers who breast fed their children for >4 months, compared with mothers who breast fed their children <4 months, were older and had a lower body mass index (Supplementary Table S2). Mothers who exclusively breast fed their children had a lower weight, were more likely to have >1 child and had children with a higher birth weight (Supplementary Table S3).

The associations of ever breastfeeding, breastfeeding duration and exclusivity with peripheral, central and total fat mass at the ages of 1.5, 6 and 24 months are shown in Table 2. Breastfeeding duration was not associated with subcutaneous fat mass at the age of 1.5 months. Shorter breastfeeding was associated with higher peripheral and total subcutaneous fat mass at the age of 6 months (*P*-value for trend <0.05), but not at the age of 24 months. As compared with children who were breast fed exclusively for 4 months, those who were never breast fed had a higher peripheral and total subcutaneous fat mass at the age of 6 months and higher central fat mass at the age of 24 months (*P*-value for trend <0.01).

Table 3 presents the associations between age at introduction of solid foods and peripheral, central and total fat mass at the ages of 1.5, 6 and 24 months. As compared with children who received solid foods after the age of 5 months, those who received solid foods before the age of 5 months, tended to have increased subcutaneous fat mass measures, but these associations were not significant.

Discussion

Main findings

Results from this study suggest that a shorter duration and non-exclusive breast fed affect early body composition during the first 2 years of life. A shorter duration of breastfeeding was associated with higher peripheral and total subcutaneous fat mass at the age of 6 months, whereas non-exclusive breast fed was associated with higher central subcutaneous fat mass at the age 24 months. Early introduction of solid foods was not associated with subcutaneous fat mass measures.

Strengths and weaknesses

The most important strengths of this study are the population-based prospective design with a relatively large number of subjects being studied from early pregnancy onwards, and information about a large number of potential confounders available. Our analyses were based on 779 children with SFT measurements. Furthermore, information was available about duration and exclusivity of breastfeeding. Some methodological issues need to be considered. Of the total

Table 2 Associations of ever breastfeeding, breastfeeding duration and exclusivity of breastfeeding with subcutaneous skinfolds (mm) at different ages in early childhood^{a,b}

	Age 1.5 months			Age 6 months			Age 24 months		
	Peripheral fat (mm) (95% CI)	Central fat (mm) (95% CI)	Total fat (mm) (95% CI)	Peripheral fat (mm) (95% CI)	Central fat (mm) (95% CI)	Total fat (mm) (95% CI)	Peripheral fat (mm) (95% CI)	Central fat (mm) (95% CI)	Total fat (mm) (95% CI)
N = 779									
Ever breast fed									
Never (n=97)	N = 653 -0.24 (-1.02, 0.54) Reference	N = 648 -0.22 (-0.92, 0.49) Reference	N = 648 -0.45 (-1.82, 0.93) Reference	N = 688 1.00 (0.10, 1.89)* Reference	N = 686 0.25 (-0.51, 1.02) Reference	N = 686 1.24 (-0.22, 2.70) Reference	N = 584 0.23 (-0.98, 1.44) Reference	N = 575 0.62 (-0.34, 1.57) Reference	N = 575 0.86 (-1.07, 2.80) Reference
Ever (n=682)									
Duration of being breast fed									
Never (n=97)	N = 615 -0.16 (-0.92, 0.62) Reference	N = 611 -0.12 (-0.80, 0.57) Reference	N = 611 -0.26 (-1.60, 1.08) Reference	N = 647 1.49 (0.51, 2.47)** Reference	N = 645 0.50 (-0.35, 1.35) Reference	N = 645 1.99 (0.37, 3.61)* Reference	N = 553 0.21 (-1.13, 1.55) Reference	N = 544 0.90 (-0.14, 1.94) Reference	N = 544 1.14 (-0.98, 3.26) Reference
<4 months (n=354)									
≥4 months (n=283)	NA	NA	N.A	(0.02, 1.37)* Reference	(-0.24, 0.92) Reference	(-0.06, 2.15) Reference	(-0.82, 0.91) Reference	(-0.17, 1.17) Reference	(-0.82, 1.92) Reference
P for trend	P = 0.38	P = 0.79	P = 0.50	P < 0.01	P = 0.18	P = 0.01	P = 0.79	P = 0.06	P = 0.26
Exclusive breast fed									
Never (n=97)	N = 653 0.46 (-0.43, 1.35) Reference	N = 648 0.14 (-0.67, 0.95) Reference	N = 648 0.58 (-0.99, 2.15) Reference	N = 688 1.40 (0.37, 2.42)** Reference	N = 686 0.57 (-0.30, 1.44) Reference	N = 686 1.96 (0.29, 3.63)* Reference	N = 584 0.50 (-0.86, 1.86) Reference	N = 575 1.24 (0.17, 2.30)* Reference	N = 575 1.75 (-0.42, 3.91) Reference
Non-exclusive until 4 months (n=472) ^c	0.96 (0.36, 1.56)** Reference	0.49 (-0.05, 1.04) Reference	1.42 (0.36, 2.48)** Reference	0.55 (-0.14, 1.24) Reference	0.44 (-0.15, 1.03) Reference	1.00 (-0.13, 2.12) Reference	0.37 (-0.49, 1.23) Reference	0.86 (0.19, 1.53)* Reference	1.23 (-0.14, 2.59) Reference
Exclusive until 4 months^c (n=210)									
P for trend	P = 0.07	P = 0.41	P = 0.16	P < 0.01	P = 0.13	P = 0.02	P = 0.38	P < 0.01	P = 0.06

^aValues are unstandardised regression coefficients (95% confidence interval (CI)).

^b*P < 0.05 and **P < 0.01 using multiple linear regression models. Models are adjusted for child's age at visit, sex, birth weight, maternal education, maternal body mass index, smoking and parity, gestational age, current height and observer.

^cNon-exclusive breastfeeding until 4 months includes partial until 4 months, partial thereafter; and partial until 4 months, not thereafter. Exclusive breastfeeding until 4 months includes exclusive until 6 months; exclusive until 4 months, partial thereafter and exclusive until 4 months, not thereafter.

Table 3 Age at introduction of solid foods and subcutaneous skinfolds (mm) in early childhood^{a,b,c}

Solid foods (fruit or vegetables) N = 779	Age 6 months			Age 24 months		
	Peripheral fat (mm) (95% CI) N = 486	Central fat (mm) (95% CI) N = 484	Total fat (mm) (95% CI) N = 484	Peripheral fat (mm) (95% CI) N = 476	Central fat (mm) (95% CI) N = 469	Total fat (mm) (95% CI) N = 469
<4 months (n = 48)	0.23 (−1.11, 1.56)	0.96 (−0.30, 2.21)	1.18 (−1.04, 3.40)	1.66 (−0.11, 3.42)	−0.09 (−1.39, 1.21)	1.29 (−1.46, 4.04)
>4–5 months (n = 378)	−0.06 (−0.79, 0.67)	0.45 (−0.24, 1.14)	0.40 (−0.82, 1.61)	0.82 (−0.09, 1.73)	0.66 (−0.01, 1.33)	1.46 (0.05, 2.88)*
>5 months (n = 200)	Reference	Reference	Reference	Reference	Reference	Reference
P for trend	P = 0.89	P = 0.10	P = 0.31	P = 0.03	P = 0.29	P = 0.08

^aValues are unstandardized regression coefficients (95% confidence interval (CI)) using multiple linear regression models.

^bModels are adjusted for child's age at visit, sex, maternal education, maternal body mass index, smoking and parity, gestational age, birth weight, current height, observer and breastfeeding duration.

^c*P < 0.05.

group of 1039 children, breastfeeding information was available in 78%. This non-response would lead to biased effect estimates if the associations of breastfeeding duration and exclusivity with SFT measurements would be different between those included and not included in the analyses. This seems unlikely because biased estimates in cohort studies mainly arise from loss to follow-up rather than from non-response at baseline (Nohr *et al.*, 2006). Information about breastfeeding was prospectively collected by questionnaires without direct reference to any skinfold measurement. Although assessing breastfeeding by questionnaires seems to be a valid method, misclassification may occur (Li *et al.*, 2005). We estimated breastfeeding exclusivity according to whether the child received breastfeeding without any other infant formula, milk or solids according to the short food frequency questionnaire. This definition does not cover the strict criteria used by the WHO (World Health Organization), which suggest that even the use of water in combination with breastfeeding does not fulfill the definition of exclusivity. However, we did ask for the most commonly introduced solids and fluids. Furthermore, in the Netherlands it is not common that children receive breastfeeding in combination with the use of water to prevent dehydration. Therefore, we consider our measurement of exclusive breast fed as a good proxy for exclusive breast fed according to the WHO criteria. Our definition of solid foods included only fruit or vegetable snacks. However in the first months of life it is not likely that other products were introduced. We created our specific duration, exclusivity and solid foods categories based on the collected data and growth measurements. These categories do not enable direct comparison with the widely used categories (6 months) of the WHO. Furthermore, our study group was ethnically homogenous and the mothers were highly educated. We were not able to assess the effect of breastfeeding on subcutaneous fat mass development in children with different ethnic and social backgrounds. Finally, we used SFT as a measure of subcutaneous fat mass because of the limited use of body mass index as a direct measure of

adiposity in early childhood. SFT provide a simple, easy and quick yet highly informative assessment of regional fatness in most age groups and can be used in large-scale epidemiological studies (Wells and Fewtrell, 2006). In general, intraobserver and interobserver error are low compared with between-subject variability, but in obese children accuracy and precision are poorer (Nevill *et al.*, 2006; Wells and Fewtrell, 2006). Furthermore, SFT has a limitation in assessing lean and fat mass of the whole body (Wells and Fewtrell, 2006).

Comparison with other studies

Several studies have shown that breastfeeding is associated with a lower risk of later overweight and obesity (Arenz *et al.*, 2004; Harder *et al.*, 2005; Owen *et al.*, 2005a, b). Body mass index is a poor outcome due to its low predictive value and lack of information about fat distribution (Wells *et al.*, 2007). Studies focusing on the association between breastfeeding and body composition instead of body mass index did show inconsistent associations (Burdette *et al.*, 2006; Gale *et al.*, 2007). This may be due to differences in body measurements, ages and samples sizes. A study among adult males from Brazil did not show an association between breastfeeding and adult body fat, measured by skinfolds and fat mass using a bio-impedance scale (Victoria *et al.*, 2003), whereas a large study in the United Kingdom reported a negligible protective effect of breastfeeding duration for >6 months on mean body fat measured with the dual-energy X-ray absorptiometry in children aged 9–10 years (Toschke *et al.*, 2007). Any association was attenuated after adjustment for confounders. One study in Southampton reported a graded association between shorter breastfeeding duration and higher DXA-derived fat mass in children aged 4 years (Robinson *et al.*, 2009). In contrast, two studies, assessing the associations of breastfeeding and direct measures of body composition using dual-energy X-ray absorptiometry at ages of 2 and 5 years, did not show any association (Tulldahl *et al.*, 1999; Burdette *et al.*, 2006). We used SFT as measures of

subcutaneous fat mass at younger ages, but the results are in line with these previous studies. Only at the age of 6 months we found that shorter breastfeeding duration leads to higher peripheral and total fat mass. Our study was the first study that used both breastfeeding duration and exclusivity, to examine the effect on subcutaneous fat mass. We showed that non-exclusive breast fed was associated with higher peripheral and total subcutaneous fat mass at the age of 1.5 months and higher central fat mass at the age of 24 months. We cannot explain these differences. However, central fat mass might be stronger related to adverse cardiovascular and metabolic health outcomes (Fox *et al.*, 2007; Pischon *et al.*, 2008). Although the effect estimates were small, exclusive breast fed might affect subcutaneous fat mass development in early childhood.

The European Society for Pediatric Gastroenterology, Hepatology and Nutrition Nutrition Committee recommended in 2008 that complementary foods (solid foods and liquids other than breastfeeding or formula) should not be introduced before 4 months and not later than 6 months (Agostoni *et al.*, 2008). Many studies in industrialized countries showed non-adherence to these recommendations (Noble and Emmett, 2006; Scott *et al.*, 2009). In our Dutch study population, we found that most mothers introduced solid foods mainly after the age of 4 months. There is conflicting evidence about the relation between timing of introduction of solid foods and adiposity in childhood. A few studies did not show differences in adiposity between early and delayed introduction of solid foods (Kramer, 1981; Zive *et al.*, 1992). One recent study suggested that a diet based on fruit, vegetables and home-prepared foods in the first year of life is associated with a higher lean and lower fat mass measured with DXA at the age of 4 years (Robinson *et al.*, 2009). The same study group reported a greater gain in both weight and SFT between 6 and 12 months among infants who received the same diet with fruit, vegetables and home-prepared foods at the age of 6 months (Baird *et al.*, 2008). We did not observe significant associations between early introduction of solid foods and subcutaneous fat mass measures.

Conclusion

Our results suggest that a shorter duration and non-exclusive breast fed affect early body composition during the first 2 years of life. Early introduction of solid foods was not associated with subcutaneous fat mass measures. Follow-up studies are needed to assess whether breastfeeding duration and exclusivity affect subcutaneous fat mass and other measures of body composition at older ages.

Conflict of interest

The authors declare no conflict of interest.

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