



Trabajo Original

Feeding patterns and growth trajectories in breast-fed and formula-fed infants during the introduction of complementary food

Patrones de alimentación y trayectorias de crecimiento en niños con lactancia materna y con leche de fórmula durante la introducción de la alimentación complementaria

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Abstract

Introduction: several investigations have identified breastfeeding as a protective factor for rapid infant weight gain and childhood obesity while other studies have found that this protective effect could be the result of confounding factors.

Objectives: to assess the associations between lactation practices (breast-fed vs formula-fed infants) during the introduction of complementary food period, as well as the following: a) patterns of food intake; and b) trajectories of growth at six, nine and 12 months (z-score of weight, height and body mass index [BMI] and changes in these z-scores from six to 12 months).

Methods: two hundred and three infants randomly selected from Spanish Primary Health Centres were measured. Parents recorded all infant's food consumption for three days (g/day). Linear regression models were applied.

Results: breast-fed infants had a lower intake of cereals, fruit baby food, vegetables with meat/fish and a total intake of food compared to formula-fed infants at nine months of age. After adjusting for sex, parental education and total food intake, breastfed children continued to have lower intake of cereals (-5.82, 95% CI: -9.22, -2.43), and lower total food intake (-301.23, 95% CI: -348.50, -253.96). Breast-fed infants had a lower change in z-score of weight, height and BMI from six to 12 months of age and these differences remained when adjusting for all confounders.

Conclusions: formula-fed infants during the complementary feeding period have a higher food intake and show higher rates of rapid infant weight gain compared to breast-fed infants. These differences in growth trajectories depending on breastfeeding maintenance and food intake during early life must be considered in adiposity risk evaluation.

Key words:

Breastfeeding.
Fórmula. Infantes.
Complementary
food. Obesity. Growth
trajectories.

Resumen

Introducción: varios estudios han identificado la lactancia materna como un factor protector frente a la ganancia rápida de peso y la obesidad infantil, mientras que otros estudios han encontrado que este efecto protector podría ser el resultado de la interferencia de factores de confusión.

Objetivos: evaluar las asociaciones entre el tipo de lactancia (leche materna versus lactantes alimentados con fórmula) durante la introducción de la alimentación complementaria, así como: a) los patrones de ingesta de alimentos; y b) las trayectorias de crecimiento a los seis, nueve y 12 meses (z-score de peso, estatura e índice de masa corporal [IMC] y variaciones en estos z-score entre los seis y los 12 meses).

Métodos: se midieron 203 neonatos seleccionados en centros de salud primaria españoles. Los padres registraron el consumo de alimentos de todos los bebés durante tres días (g/día). Se aplicaron modelos de regresión lineal.

Resultados: los bebés alimentados con leche materna tuvieron una menor ingesta de cereales, alimentos para bebés a base de frutas, verduras con carne/pescado, así como una menor ingesta total de alimentos en comparación con los bebés alimentados con fórmula a los nueve meses de edad. Después de realizar el ajuste por sexo, educación de los padres y consumo total de alimentos, los niños alimentados con leche materna continuaron teniendo una menor ingesta de cereales (-5,82, IC 95%: -9,22, -2,43) y una ingesta total de alimentos más baja (-301,23, IC 95%: -348,50, -253,96). Los bebés alimentados al pecho tuvieron un menor incremento en la puntuación z-score de peso, talla e IMC entre los seis y los 12 meses de edad y estas diferencias se mantuvieron cuando se ajustaron para todos los factores de confusión.

Conclusiones: los lactantes alimentados con fórmula durante el periodo de alimentación complementaria tienen una mayor ingesta de alimentos y muestran tasas más altas de ganancia de peso en comparación con los lactantes alimentados con leche materna. Estas diferencias en las trayectorias de crecimiento en función del mantenimiento de la lactancia materna y la ingesta de alimentos durante los primeros años de vida deben considerarse en la evaluación del riesgo de adiposidad.

Palabras clave:

Lactancia materna.
Fórmula. Lactantes.
Alimentación
complementaria.
Obesidad.
Trayectorias de
crecimiento.

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INTRODUCTION

Breastfeeding has been suggested to exert a protective effect on childhood obesity (1,2). However, the evidence in the literature for this relationship is still inconsistent and some studies have indicated that this association could be influenced by publication bias and confounding factors (3,4). Particularly, in high income countries, a strong social gradient is observed for breastfeeding and mothers with a higher socioeconomic status (SES) are more likely to breastfeed and for a longer period (5). Moreover, they are less likely to smoke during the pregnancy and to be obese and both factors are strong predictors of childhood obesity (6).

More consistent findings have been reported between formula-fed infants and rapid infant gain weight (7). Formula-fed infants gained weight more rapidly out of proportion to length than breast-fed infants (8) during the first year of life, which results in a higher BMI and therefore, a higher risk for future obesity. It has been stated that breastfeeding may promote self-regulation on an infant's energy intake (2). Furthermore, some studies have found that there are hormones, growth factors or bioactive substances that are in breast milk and not in formula that could inhibit adipogenesis (9,10) and consequently, prevent future childhood obesity.

Other possible mechanism explaining differences between breast-fed and formula-fed infants has been that a longer duration of breastfeeding delays the introduction of complementary feeding (11), which is associated with an increased infancy weight gain when the introduction of solid foods is before four months (12). Earlier introduction of complementary food might lead to higher energy intakes from an earlier age since breast milk is low in protein and could be replaced by complementary higher in energy and protein (13).

Only when breast milk is no longer enough to meet the nutritional needs, complementary foods should be introduced to the diet of the child, typically after six months of age according to the World Health Organization (WHO) guidelines (14).

Some studies have reported that the energy and protein intakes in formula-fed infants seem to be higher than in breast-fed children (15). However, to the best of our knowledge, no studies have assessed the total intake (g/day) of various food groups (cereals, fruit baby food, vegetables with proteins such as meat or fish baby food, formula and yogurt) in formula-fed and breast-fed infants during the introduction of complementary food combined with the assessment of growth trajectories in both groups.

We hypothesized that formula-fed infants have a higher intake of each food group and, therefore, formula-fed infants have a higher BMI compared to breast-fed infants. The present paper aims to explore the associations: a) between lactation practices (breast-fed vs formula-fed infants) and feeding patterns during the introduction of complementary food period; and b) between lactation practices and trajectories of growth by measuring weight z-score, height z-score and BMI z-score (at six, nine and 12 months of age); as well as changes occurred in weight z-score, height z-score and BMI z-score from six to 12 months of age in a cohort of children from Spain.

METHODS

DESIGN AND STUDY POPULATION

The present study included a cohort of children from different localities in Spain randomly selected in Primary Care Centers within the frame of a project financed by the Centre for the Development of Industrial Technology (CDTI) (NEOBEFOOD project, IDI-20100569). In particular, the cohort included all children without any malformation, diseases or physical disabilities that could affect growth and nutritional status. They were recruited from Primary Care Centers in the Spanish Child Health Program, participated from March to July 2011 in different localities in the north of Spain (located in the province of Huesca and Santander) and were followed for one year (from six months of age to 18 months of age). These localities were chosen because the participating research groups had not only logistic capacity due to geographic proximity but also, they had previous experience in the area and some of the research members were working in the Primary Care Centers studied. The main objective of the study was to assess growth patterns, body composition, digestive conditions (digestive tolerance, satiety and depositions), and feeding aspects of infants and children, and to examine prenatal, postnatal and socio-cultural factors which may influence them.

Permanent trained pediatric staff conducting the Spanish Child Health Program at least in the last two years before being included in the study.

To calculate the sample size needed for the present study, the variance of weight was considered as the best anthropometric variable related to the nutritional status during the first months of life. For the present study, the weight variance was chosen at 18 months of age (1.26 kg), that is, the last moment of follow-up for the chosen sample, using WHO reference data (16). A similar size of the two similar groups and similar variables of weight at 18 months, risk of type I error of 5% ($\alpha = 0.05$) and power of 80% ($\beta = 0.20$) were considered. From the previous data, a sample size of 200 children was obtained, but taking into account that 20% of the children who started the study would be lost, would change their address or would abandon, the final sample was established in 240. In conclusion, to achieve a power of 80%, with a confidence level of 95%, and to demonstrate a difference in weight of 0.5 kg at 18 months of age and assuming losses of 20%, 120 subjects in each group (formula and breast-fed infants) were needed, that is, 240 subjects.

Finally, 272 families were contacted to participate in the study, of which 245 accepted to participate (acceptance rate 90%). These 246 new-born infants without any malformation, diseases or physical disabilities were examined at six months of age and periodically re-examined in Primary Care Centers at six, nine and 12, 15 and 18 months of age. After one year of follow-up, 61 children did no longer participate in our study, thus the retention rate was 75%. Children with missing values in any of the exposures, covariates or outcomes because they did not come to the follow-up examinations, did not continue with the study or changed their home address, or did not provide the required

information were excluded (43 children). In the end, the analysis included 203 children (55.3% boys).

Parents or legal guardians gave written informed consent for examinations for their children. Ethical approval was obtained from the Committee of Ethics in Clinical Research (CEICA) of Aragón.

MEASUREMENTS

Outcome measure (feeding patterns at nine months)

A 24-hour dietary recall interview conducted at the Primary Care Centers was used to record all infant's food consumption (in grams or cubic centimeters [cc] a day). During the interview, parents were asked about the intake of: a) *cereals* (single grain cereals in powder to be mixed with water or formula); b) *fruit baby food*; c) *vegetables with meat or fish baby food*; d) *formula*; and d) *yogurt* before the programmed visits at nine months of age.

To avoid misreport and reduce key factors associated with bias, the 24-hour diet recall was carried out by a previously trained interviewer on dietary assessment with a thorough procedure protocol.

The total intake of grams was calculated by summing the quantity of cereals, fruit baby food, vegetables with proteins, baby food and yogurt in g/day (excluding formula and breast milk). Those food items reported in cubic centimeters were converted into grams.

In the present study we have focused on infants at the age of nine months because during this period the transition from exclusive breastfeeding to family foods, referred to as complementary feeding, should have already been established with the consumption of a large variety of foods.

Secondary outcome measure (trajectories of growth)

Height and weight were measured by trained staff using the homologated measuring Seca® 374 medical digital baby scale. Child length was obtained using a recumbent board to the nearest 0.1 cm and body weight in kg to the nearest 10 g in a fasting state and without any clothes. We calculated age- and sex-specific weight, height, weight/length ratio and BMI z-scores using the WHO reference data (16). Changes in weight z-score, height z-score and BMI z-score were calculated as the difference between 12 months and six months of age z-score of weight, height and BMI respectively.

Exposure measure (lactation practices)

Mothers were asked about their children feeding practices at the age of nine months. Specifically, mothers reported the duration of exclusive breastfeeding, weaning time, introduction of formula

feeding and intake of each food group during the introduction of complementary food. Nine months of age is a representative period to quantify food and to assess infant food patterns. At this age infants eat a variety of food that can be easily assessed because they are usually in a liquid or smashed status that before (i.e., at six months) or after (i.e., at nine months) will not be possible.

With this information, children were categorized as breast-fed (including children who were both breast-fed and formula-fed) and formula-fed infants (children who were just formula-fed).

Potential confounding factors

Parental education

By an individual face-to-face interview with parents, data regarding their educational level were collected. Mothers and fathers were asked to indicate their highest level of education. Particular response categories were coded according to International Standard Classification of Education (ISCED 1997) (17) and re-categorized into three categories: low (0-2), medium (3-4) and high (5-6) ISCED educational levels.

Parental BMI

Paternal and maternal BMI were calculated from fathers' and mothers' (pre-pregnancy) weight and height reports. This information was obtained by a face-to-face interview with parents.

Statistical analyses

We assessed whether infant's food consumption (primary outcome) and trajectories of growth (secondary outcome) differed according to our covariates and exposures significantly using t-test for dichotomous variables (sex and lactation practices) and analysis of variance (ANOVA) for categorical variables (maternal and paternal education).

Additionally, linear regression models were applied to assess the associations between lactation practices at nine months (breast-fed vs formula-fed infants) and each primary outcome (intake of cereals, fruit baby food, vegetables with proteins such as meat or fish, formula and yogurt and total food intake in g/day) at nine months and secondary outcome (z-score of weight, height, BMI and changes in these anthropometry data for the period occurred from six to 12 months). The reference category used was formula-fed infants.

To adjust for possible confounders, two different models were run for the primary outcome. Model 1 was adjusted for sex, maternal education and paternal education. In the final adjusted model (model 2), we additionally adjusted for children's total intake (excluding formula and breast milk) by summing the quantity of cereals, fruit baby food, vegetables with meat and fish and baby food in g/day. Due to the difficulty of estimating the precise

amount of breast milk consumed, the intake of formula and breast milk was excluded in the final adjusted models to better compare the total intake in g/day of breast-fed and formula-fed children.

To assess the trajectories of growth at six, nine and 12 months (secondary outcome) in breastfed and formula-fed infants (at nine months), models were adjusted for sex, parental education and parental BMI (model 1). In the final adjusted model (model 2), we additionally adjusted for children's total intake (excluding formula and breast milk).

Models assessing the changes in z-score of weight, height and BMI from six to 12 months models were adjusted for sex, parental education, parental BMI and children's weight at six months in basic models (model 1). In the final adjusted model (model 2), we additionally adjusted for children's total intake (excluding formula and breast milk).

Significance level was set at 0.05. Analyses were performed using the Statistical Package for the Social Sciences (version 22.0; SPSS, Inc.).

RESULTS

Table I summarizes children's feeding intake (in g/day) at age nine months based on children's sex, lactation practices and parental education. Mean and standard deviation (SD) are shown. In the present study, most of the children were formula-fed at nine months of age. By sex, the mean of children food intake was lower in females than in males. Children who were breast-fed had also a lower mean intake of cereals, fruit baby food, vegetables with meat/fish baby food, formula, and a total intake of food when excluding formula and breast milk as compared to those infants who were formula-fed.

Table II shows children's weight, height and BMI (in z-score) at nine months and changes in anthropometry occurred (z-score of weight, height and BMI) from six to 12 months based on children's sex, lactation practices, parental education and parental BMI. Mean and standard deviation (SD) are shown. See supplementary table S1 for information at age six and 12 months.

Table I. Children's feeding intake (in g/day) at nine months of age based on children's sex, lactation practices and parental education. Mean and standard deviation (SD) are reported

n = 203 (100%)	Cereals* (g/day)	Carbohydrates† (g/day)	Fruit baby food (g/day)	Vegetables with meat/fish baby food (g/day)	Formula (g/day)	Yogurt (g/day)	Total intake excluding formula and breast milk (g/day)*
<i>Sex</i>							
Male (44.7%)	24.31 (9.07)	17.70 (30.39)	209.66 (73.81)	258.74 (91.04)	479.20 (156.05)	156.02 (58.18)	466.98 (268.30)
Female (55.3%)	23.56 (12.23)	11.50 (12.88)	185.82 (73.65)	250.60 (94.77)	427.47 (185.04)	162.77 (69.52)	440.33 (244.84)
p-value	0.638	0.456	0.028	0.536	0.037	0.579	0.422
<i>Paternal education</i>							
Low (22.8%)	0.73 (8.77)	15.82 (14.54)	191.03 (74.30)	272.33 (106.32)	461.750 (199.44)	166.34 (977.62)	438.31 (282.18)
Medium (39.8%)	24.84 (12.39)	24.77 (39.69)	210.13 (72.64)	255.51 (94.20)	459.87 (151.78)	160.71 (69.21)	452.96 (269.57)
High (48.4%)	23.77 (9.31)	6.77 (9.68)	192.10 (75.99)	245.49 (82.55)	449.73 (175.40)	152.56 (57.42)	467.51 (230.55)
p-value (37.4%)	0.580	0.209	0.254	0.309	0.913	0.663	0.797
<i>Maternal education</i>							
Low (11.8%)	21.73 (8.38)	21.00 (17.55)	204.52 (80.31)	262.50 (101.13)	459.52 (192.08)	152.78 (55.12)	431.33 (256.24)
Medium (39.8%)	24.10 (11.02)	10.33 (12.55)	190.94 (64.84)	256.58 (102.25)	458.40 (187.17)	163.46 (65.10)	418.42 (272.87)
High (48.4%)	24.33 (10.57)	15.66 (31.44)	203.33 (79.63)	252.21 (83.36)	453.95 (154.00)	156.90 (63.51)	491.02 (242.39)
p-value	0.612	0.660	0.536	0.874	0.982	0.841	0.103
<i>Lactation practices</i>							
Breast-fed infant (27.1%) [§]	19.18 (9.92)	14.75 (16.75)	175.88 (76.85)	223.33 (84.81)	266.86 (121.41)	171.87 (61.81)	477.32 (176.73)
Formula-fed infant (72.9%)	25.55 (10.28)	14.67 (26.93)	207.47 (72.03)	266.65 (92.87)	510.97 (141.82)	154.53 (63.05)	560.09 (181.82)
p-value	< 0.001	0.992	0.009	0.003	0.000	0.255	0.004

*Multi grain cereals in powder to be mixed with water or formula. †Non-powder carbohydrates such as potatoes, bread or biscuits. ‡Sum of cereals, carbohydrates, fruit baby food, vegetables with meat/fish baby food and yogurt in grams/day. §Breast-fed children were defined as those children who were both breast-fed and formula-fed. ||Formula-fed infants were defined as those children who were just formula-fed.

Table II. Children's weight, height and BMI (in z-score) at nine months of age and changes in anthropometry occurred (z-score of weight, height and BMI) from six to 12 months based on children's sex, lactation practices, parental education and parental BMI. Mean and standard deviation (SD) are reported

n = 203 (100%)	Weight z-score at 9 months	Height z-score at 9 months	BMI z-score at 9 months	Changes in weight z-score from 6 to 12 months	Changes in height z-score from 6 to 12 months	Changes in BMI z-score from 6 to 12 months
<i>Sex</i>						
Male (44.7%)	0.26 (0.98)	0.27 (1.09)	0.15 (0.90)	0.40 (0.54)	0.05 (0.61)	0.50 (0.66)
Female (55.3%)	0.32 (0.81)	0.34 (0.87)	0.17 (0.91)	0.35 (0.45)	0.04 (0.62)	0.46 (0.54)
p-value	0.610	0.595	0.864	0.534	0.851	0.641
<i>Paternal education</i>						
Low (22.8%)	0.50 (1.02)	0.47 (1.18)	0.32 (0.90)	0.38 (0.53)	0.04 (0.60)	0.49 (0.61)
Medium (39.8%)	0.32 (0.90)	0.40 (0.96)	0.13 (0.92)	0.41 (0.49)	0.09 (0.64)	0.50 (0.61)
High (48.4%)	0.14 (0.83)	0.12 (0.90)	0.10 (0.90)	0.34 (0.51)	0.01 (0.60)	0.45 (0.61)
p-value	0.097	0.092	0.376	0.639	0.732	0.850
<i>Maternal education</i>						
Low (11.8%)	0.17 (0.93)	0.44 (1.28)	-0.10 (1.00)	0.39 (0.48)	0.19 (0.63)	0.39 (0.58)
Medium (39.8%)	0.25 (0.85)	0.25 (0.99)	0.14 (0.87)	0.38 (0.51)	0.05 (0.59)	0.48 (0.56)
High (48.4%)	0.35 (0.93)	0.31 (0.97)	0.23 (0.90)	0.37 (0.50)	0.02 (0.63)	0.50 (0.65)
p-value	0.612	0.660	0.536	0.874	0.982	0.841
<i>Paternal BMI</i>						
Normal weight* (62.5%)	0.28 (0.90)	0.19 (14.54)	0.23 (0.87)	0.31 (0.51)	-0.01 (0.64)	0.44 (0.67)
Overweight (29.3%)	0.27 (0.93)	0.40 (0.89)	0.06 (0.96)	0.42 (0.49)	0.07 (0.54)	0.52 (0.58)
Obesity (8.2%)	0.40 (0.92)	0.42 (0.96)	0.23 (0.93)	0.43 (0.45)	0.10 (0.56)	10.56 (57.42)
p-value	0.821	0.307	0.454	0.374	0.576	0.735
<i>Maternal BMI</i>						
Normal weight* (62.5%)	0.35 (0.85)	0.32 (0.95)	0.82 (0.07)	0.38 (0.51)	0.00 (0.66)	0.52 (0.62)
Overweight (29.3%)	0.21 (1.03)	0.30 (1.07)	1.07 (0.13)	0.42 (0.49)	0.13 (0.51)	0.48 (0.58)
Obesity (8.2%)	0.08 (0.90)	0.09 (1.13)	0.96 (0.23)	0.25 (0.11)	0.10 (0.59)	6.28 (0.61)
p-value	0.374	0.668	0.392	0.462	0.435	0.334
<i>Lactation practices at 9 months of age</i>						
Breast-fed infant (27.1%)	0.33 (0.96)	0.40 (0.90)	0.13 (1.10)	0.15 (0.50)	-0.13 (0.73)	0.30 (0.58)
Formula-fed infant (72.9%)	0.29 (0.89)	0.28 (1.04)	0.18 (0.83)	0.46 (0.48)	0.11 (0.56)	0.55 (0.62)
p-value	0.798	0.446	0.700	< 0.001	0.018	0.014

*Normal weight includes those parents underweight and normal weight.

In the present study, most of the children were formula-fed at age nine months. No significant mean differences were found between sex, parental BMI and parental education and variables of anthropometry studied. During this period, children who were breast-fed had lower changes in weight z-score, height z-score and BMI z-score, as compared to those infants who were formula-fed. Weight length ratio was also calculated, but a similar pattern was found.

Table III presents beta coefficients (β) and 95% CI for the associations between breastfeeding status and each food group intake (g/day) at nine months. In the basic models (adjusted for sex and parental education), breast-fed practices were associated with a lower intake of cereals, fruit baby food, vegetables with proteins baby food, formula milk and total intake of food (excluding formula and breast milk) in comparison to formula-fed infants at nine months. In the fully adjusted models, only associations between breast-fed infants and lower intake of cereals, between breast-fed infants and lower intake of formula and between breast-fed infants and lower total intake of food remained significant.

Table IV shows the associations between changes in z-score of weight, height and BMI from six to 12 months and lactation practices at nine months of age. After adjusting for sex, parental education, parental BMI and children's weight at six months, breast-fed infants had a lower change in z-score of weight, height

and BMI. When models were additionally adjusted for children's total intake (model 2, fully adjusted models), associations remained significant for lactation practices at nine months of age and z-score of weight and height from six to 12. Associations between lactation practices at nine months of age and z-score of BMI from six to 12 were no longer significant but associations pointed to the same direction.

In additional analyses, the associations between breastfeeding status at nine months of age and children's anthropometry (z-scores of weight, height and BMI) at six, nine and 12 months of age, respectively, were studied. However, no statistically significant associations were children's anthropometry and breastfeeding status (results not shown). Further analyses were conducted by including not only BMI but also weight to length ratio. Nevertheless, the weight (in kg) to length (in cm) ratio yielded similar results, therefore only results with BMI are displayed.

DISCUSSION

This investigation studied the association between: a) lactation practices (breast-fed vs formula-fed infants) and feeding patterns during the introduction of complementary food period; and b) lactation practices and trajectories of growth by measuring weight

Table III. Associations between breastfeeding status and each food group intake (g/day) at nine months of age

Breastfeeding status at age 9 months	M1*			M2†		
	β	95% CI	p-value	β	95% CI	p-value
Intake of cereals at age 9 months						
Breast-fed infant	6.26	9.61	< 0.001	-5.82	9.22	0.001
Formula-fed infant		2.91			2.43	
Intake of fruit baby food at age 9 months						
Breast-fed infant	27.97	52.05	0.023	6.99	26.61	0.483
Formula-fed infant		3.89			12.64	
Intake of vegetables with meat/fish baby food at age 9 months						
Breast-fed infant	41.49	72.58	0.005	10.55	33.58	0.367
Formula-fed infant		12.39			12.47	
Intake of formula at age 9 months						
Breast-fed infant	241.52	288.69	< 0.001	-251.68	298.88	< 0.001
Formula-fed infant		194.34			204.52	
Total food intake at age 9 months						
Breast-fed infant	368.59	432.51	< 0.001	301.23	348.50	< 0.001
Formula-fed infant		304.67			253.96	
Intake of yogurt at age 9 months						
Breast-fed infant	18.03	11.45	0.228	0.05	0.06	0.387
Formula-fed infant		47.50			0.14	

Results from the linear regressions models*. Beta coefficients (β), 95% confidence intervals (CI) and p-values are shown. Statistically significant results are shown in italics. *Model 1 was adjusted for sex, maternal and paternal education. †Model 2 was additionally adjusted for total food intake in g/day (sum of cereals, carbohydrates, fruit baby food, vegetables with proteins, baby food excluding infant formula and breast milk).

Table IV. Associations between changes in z-score of weight, height and BMI from six months to 12 months and lactation practices at nine months of age

Breastfeeding status at age 9 months	Changes in z-score of weight			Changes in z-score of height			Changes in z-score of BMI								
	M1*			M1*			M1*								
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value						
	M2†	M2†	M2†	M2†	M2†	M2†	M2†	M2†	M2†						
Breast-fed infant	0.28	0.44	< 0.001	0.25	0.41	0.002	0.24	0.44	0.021	0.21	0.41	0.035	0.19	0.39	0.064
Formula-fed infant		0.12			0.09			0.04			0.00		0.02	0.01	

Results from the linear regressions models. Beta coefficients (β), 95% confidence intervals (CI) and p-values are shown. Statistically significant results shown in italics. †All models were adjusted for sex, parental education, parental BMI and children's weight at six months. ‡Model 2 was additionally adjusted for total food intake in g/day (sum of cereals, carbohydrates, fruit baby food, vegetables with proteins, baby food excluding infant formula and breast milk).*

z-score, height z-score and BMI z-score (at six, nine and 12 months of age); as well as changes occurred in weight z-score, height z-score and BMI z-score from six to 12 months of age in a cohort of children from Spain.

We found that the total food intake was lower in breast-fed infants as compared to formula-fed infants at nine months of age. More specifically, breast-fed children had about 300 g less a day than breast-fed infants after adjusting for sex, parental education and intake of food excluding breastfeeding and formula intake. These differences were mainly due to a lower intake of cereals and formula milk that breast-fed infants consumed when compared to formula-fed infants. The consumption of powder cereal is a common practice among Spanish children. These cereals are usually added to water or milk (breast milk or formula).

We also found that at nine months of age infants intake is mainly based on formula, fruit baby food and vegetables with meat and fish baby food in both breast-fed and formula-fed infants.

This result might help to explain differences found in rapid infant weight gain between breast-fed and formula-fed infants during the complementary feeding period (18). During this time, children are nutritionally vulnerable, and it is also a time where life-long eating habits may be established. Therefore, it constitutes a critical period for children (19).

According to some studies, differences in the intake between breast-fed and formula-fed infants could be due to the different experience of hunger and satiation in milk composition (20). Particularly, the amount of free amino acids such as glutamate, which have been associated with satiation, is much higher in breast milk than in formula (21). Thus, higher levels of glutamate in human breast milk may enhance satiety and be partly responsible for a lower weight gain in breast-fed infants (22). The number of bioactive nutrients is also different in breast milk and formula, and some of the hormones present in breast milk (such as adiponectin) may contribute to a lower incidence of future childhood obesity (23). Heinig et al. (24) found that energy intake as well as protein intake were greater in formula-fed than in breast-fed children. According to this study, gains in weight and lean body were lower in the breast-fed groups than in the formula-fed group.

Wright et al. (25) also found that the feeding method (breastfeeding or formula) impacts on feeding behaviors and hunger patterns. In addition, breast-fed infants exhibited pauses while they were sucking whereas formula-fed infants sucked in a continuous way.

This study also found that there was no statistically significant association between lactation practices and children's anthropometry (weight z-score, height z-score and BMI z-score) at six, nine and 12 months. However, when comparing the changes occurred in children's anthropometry from six to 12 months, breast-fed infants had slower patterns of growth (in weight, height and BMI) compared to formula-fed infants, which is in agreement with previous studies (26,27). Associations between lactation practices at nine months of age and z-score of weight from six to 12 and z-score of height from six to 12 remained significant after adjusting for sex, parental education, parental BMI and children's weight at six months and children's total intake. This result suggests that children who are breastfed during the complementary feeding period have a lower food intake than formula-fed children but also pointed to the effect that breast milk may have in satiety.

The rapid infant weight gain showed by formula-fed infants has been associated in several studies with subsequent obesity in children due to metabolic programming (28,29).

These results would be then consistent with ours, since the different patterns found in breast-fed and formula-fed infants as well as the different composition of breast milk and formula would result in a lower intake of food by breast-fed children as compared to formula-fed children, and therefore a lower probability of developing later obesity (1).

A special strength of the present study is the fact that, to our knowledge, no research has been carried out to date concerning the association between lactation practices and feeding patterns measured in grams a day and including trajectories of growth in a Spanish cohort of children. Moreover, infant food intake at nine months of age can be easily measured in grams since most of the food is mashed. As a consequence, to avoid unnecessary bias, in the current study the overall quantity of food was measured for convenience according to the number of grams a day instead of the amount of energy (the number of kilocalories [kcal]).

These findings must be viewed critically, as this is a study with several limitations. Firstly, the sample size was calculated to be representative of a limited geographical area of Spain.

Table S1. Children's weight, height and BMI (in z-score) at six and 12 months of age based on breastfeeding practices (breast-fed vs formula-fed infants)

n = 203 (100%)	Weight z-score at age 6 months	Height z-score at age 6 months	BMI z-score at age 6 months	Weight z-score at age 12 months	Height z-score at age 12 months	BMI z-score at age 12 months
Breastfeeding practices						
Breast-fed infants (27.1%)	0.21 (0.88)	0.47 (0.85)	-0.07 (1.04)	0.39 (0.88)	0.31 (0.86)	0.28 (1.09)
Formula-fed infants (72.9%)	0.02 (0.87)	0.22 (1.02)	-0.14 (0.90)	0.47 (0.88)	0.33 (1.07)	0.39 (0.77)
p-value	0.158	0.114	0.644	0.584	0.907	0.439

Mean and standard deviation (SD) are reported.

Therefore, the conclusions on feeding patterns and growth trajectories in breast-fed and formula-fed infants of the study may not be representative from one region to another. Secondly, a selection bias cannot be precluded as most of the parents participating in this study belonged to high SES (almost half of the parents had a high education) as this study was voluntary, and usually low SES populations are less likely to take part in research (30). A further limitation is the reliance on self-reported measures (e.g., infant intake of the different food items reported by parents). Moreover, incomplete reports of food and beverage consumption may also contribute towards reporting bias. Finally, the amount of breast milk ingested was not registered due to the difficulties in assessing it.

Future lines of research could focus on the possible underlying mechanisms that can explain the results found, which could not be investigated in the current paper. Despite the fact that biological factors can be important (such as the possible influence of breast milk on gastric emptying, appetite hormones and macronutrients), other cultural and psychosocial factors such as parenting styles can also play an important role.

CONCLUSION

During the complementary feeding period, formula-fed infants might have higher food intake levels and may have higher rates of rapid infant weight gain as compared to breast-fed infants. These differences in growth trajectories depending on breastfeeding maintenance and food intake during early life must be considered in adiposity risk evaluation.

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CONFLICT OF INTERESTS

María Rodríguez-Palmero and Montserrat Rivero are employees of Laboratorios Ordesa S.L. The other authors affirm that there is no potential conflict of interest and that there were no financial ties to products in the study.

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