Longitudinal patterns of breastfeeding and its association with adiposity and subjective indicators of satiety/appetite in the first 2 years of life.

Ivonne Ramírez Silva[1], Ana Carolina Ariza[1], Sofía Barragán-Vázquez[1], Melanie Mendoza Jiménez[1], Eduardo Ortiz-Panozo [2], Carolina Batis[3], Ana Burguete-García[4], Laura Ávila-Jimenez[5], Juan A. Rivera[* 1]

[1]Center for Nutrition and Health Research, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico. Av. Universidad 655, Col. Santa María Ahuacatitlán. Cerrada los pinos y caminera. C.P. 62100, Cuernavaca, Morelos, Mexico.

[2]Center for Population Health Research, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico. Av. Universidad 655, Col. Santa María Ahuacatitlán. Cerrada los pinos y caminera. C.P. 62100, Cuernavaca, Morelos, Mexico.

[3]National Council for Science and Technology (CONACYT), Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico. Av. Universidad 655, Col. Santa María Ahuacatitlán. Cerrada los pinos y caminera. C.P. 62100, Cuernavaca, Morelos, Mexico.

[4]Center for Research in Infectious Diseases, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico. Av. Universidad 655, Col. Santa María Ahuacatitlán. Cerrada los pinos y caminera. C.P. 62100, Cuernavaca, Morelos, Mexico.

[5]Coordinación Auxiliar Médica de Investigación en Salud, Delegación Estatal Morelos, Instituto Mexicano del Seguro Social, Mexico.

***Correspondence:**

Juan A. Rivera Dommarco

jrivera@insp.mx

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Abbreviations

ASM: Appetite-Satiety Mechanisms

CBF: Continued Breastfeeding.

CEBQ: Child Eating Behaviour Questionnaire

DOHaD: Developmental Origins of Health and Disease

EBF: Exclusive Breastfeeding.

IMSS: Mexican Social Security Institute

ZBMI: Z score of Body Mass Index-for-age

ZLEN: Z score of Length-for-Age

ZWFL: Z score of Weight-for-Length

Abstract (279)

Evidence about the association between breastfeeding and its duration with growth, appetite and satiety indicators, and adiposity in low and middle-income countries facing nutritional transition is scarce. The aim of this study was to evaluate the association between longitudinal patterns of breastfeeding (exclusive [EBF] and continued [CBF]) with adiposity and growth, and the mediating role of appetite and satiety indicators in these associations in Mexican children during the first 2 years of life. Information from 378 mother-child pairs from the MAS-Lactancia birth cohort was analysed. Information was collected at birth and at months 1, 3, 6, 9, 12, 18 and 24 of life. Duration of EBF and CBF (beyond 6 months) was computed. Linear mixed models were used to assess the association of EBF and CBF with growth and adiposity. Path analysis was used for mediation analysis. Compared with the reference group (EBF duration <1 month), males with >3 to ≤6 months of EBF had less abdominal circumference (β= -0.66, p=0.05), Z-score weight-for-length (β= -0.17, p=0.19) and length-for-age (β= -0.49, p<0.01). Participants without CBF had higher BMI Z-score (β= 0.19, p<0.01), abdominal circumference (β= 0.62, p<0.01) and skinfold sum (β= 0.80, p=0.09). For EBF, mediation was confirmed for satiety responsiveness on the association with BMI Z-Score, for food fussiness for the association with abdominal circumference and length-for-age Z-score, and enjoyment of food on the association with length-for-age Z-score. For CBF, mediation was confirmed for food fussiness in the association with length-for-age. This study suggests that a longer exposure to EBF and CBF is associated with lower adiposity and better growth in children under 2 years of age, and that this association could be partially mediated by appetite and satiety indicators.

1. Introduction

Overweight and obesity affect 38.2 million children under 5 years globally (World Health Organization, 2021). Mexico is the ninth country with the highest prevalence of obesity in children and adolescents among countries of the Organization for Economic Cooperation and Development (OECD) (OECD (2019), n.d.).

According to the Developmental Origins of Health and Disease (DOHaD), alterations during early postnatal stages may increase susceptibility to develop obesity and chronic diseases throughout the life course (Zambrano & Nathanielsz, 2013). Gestation (Chen et al., 2009; Férézou-Viala et al., 2007) and the first two years of life (the first 1,000 days) are critical for the innate modulation of the appetite-satiety mechanisms (ASMs). Early feeding is an important regulator of growth and body composition (Isselmann DiSantis et al., 2011), since it occurs during a period of biological plasticity and of behavioural modelling, which can determine long-term eating habits, growth outcomes and future metabolic responses (Ramirez-Silva et al., 2015).

Epidemiological studies and meta-analysis have documented associations between infant feeding (breastfeeding or formula and complementary feeding characteristics) and the risk of obesity (Günther et al., 2007; Quah et al., 2015; Rydell et al., 1995). One of the mechanisms that have been proposed to explain this association is through alterations in the ASMs. Breastfeeding has been associated with beneficial effects in early modulation of the ASMs through its content of leptin, adiponectin, and some amino acids due to their role in the self-regulation of infant breast milk consumption (Newburg et al., 2010). Also, breastfed infants have greater opportunities of self-regulating the amount of milk than formula-fed infants (Li et al., 2010), and the act of breastfeeding implies lower parental control on feeding, which is also associated with higher satiety responsiveness in children (Shrivastava & Swati, 2023). In this regard, a previous study reported that bottle-fed infants are 67% less likely to have high satiety responsiveness compared to breastfed infants (Isselmann DiSantis et al., 2011). Moreover, previous studies have reported that satiety responsiveness, slowness in eating, and food fussiness have a negative association with children’s weight or adiposity, while food responsiveness, enjoyment of food, emotional overeating, and desire to drink are positively associated (Calderón García et al., 2023; Webber et al., 2009). Therefore, absence or short duration of breastfeeding, along with formula feeding and the early introduction of several foods (such as infant cereals, fruits, vegetables, and sugar-sweetened beverages) can modify ASMs, leading to overconsumption of foods, which in turn can influence growth and adiposity (Hernández Cordero et al., 2018; Liem & Mennella, 2002). Even though sexual dimorphism in growth and adiposity have been reported in the first months of life (since birth), with males having lower adiposity accumulation and higher growth than females (Davis et al., 2019; German & Hochberg, 2020), it is not known whether sex modifies the above association.

Currently, there is insufficient evidence regarding the mechanisms of the association between infant feeding and obesity. Previous animal and epidemiologic studies have shown that the regulation of appetite and satiety hormones by human milk may be involved, still, there is scarce information regarding the mechanisms of this association (Gridneva et al., 2016; Larson-Meyer et al., 2021). In addition, a life-trajectory approach is fundamental to consider the feeding history and growth of the child, which are highly interrelated since most of the studies have evaluated infant feeding only at one point in time. Furthermore, studies with reliable longitudinal infant feeding data that explores the associations between infant feeding at early stages with adiposity and growth are scarce in middle-income countries, which are currently experiencing a nutrition transition and are facing a growing prevalence of overweight and obesity.

Thus, the aim of this study was to evaluate the association between longitudinal patterns of breastfeeding (exclusive breastfeeding and continued breastfeeding) with adiposity and growth, and the mediating role of indicators of appetite and satiety in these associations in Mexican children during the first 2 years of life.

2. Methods

2.1 Study population

We analysed data of 378 mother-child pairs from MAS-Lactancia birth cohort with complete information about feeding practices, subjective indicators of satiety/appetite, and anthropometric measurements of children from birth to 24 month of age (**Figure 1**), which represented 49.5% of the total mother-child pairs that are still active in the cohort. A detailed description of the birth cohort protocol has been published (Ramirez-Silva et al., 2021). The study began in March 2016, so far 1210 women have been recruited at two Clinics from the Mexican Social Security Institute (IMSS for its acronym in Spanish) in Cuernavaca, Morelos. Inclusion criteria were: 18-39 y of age, at 16 to 22 weeks of gestation. Exclusion criteria included: diagnosis of hypertension, preeclampsia, renal, hepatic, or cardiovascular diseases preterm birth (<37 weeks' gestation), multiple pregnancy, evidence of maternal substance abuse, congenital diseases in children that could affect feeding and growth (i.e., cleft lip and palate, food allergies) and physical malformations which could affect anthropometric measurement. All pregnant women enrolled in the study were offered breastfeeding counselling from week 34 of gestation onwards through a series of face-to-face sessions and printed materials as part of an intervention to increase the prevalence of exclusive breastfeeding in the cohort.

The study was approved by the Human Research and Ethics Committees at the National Institute of Public Health and IMSS in Mexico. Written informed consent were obtained from all women at each stage of the study.

2.2 Study variables

2.2.1 Patterns of breastfeeding

Breastfeeding practices at 1, 3, 6, 9, 12, 18 and 24 months of age were obtained through a validated questionnaire which used two methodologies: *status-quo* and recall as recommended by the World Health Organization (WHO) (Ramirez-Silva et al., 2021; World Health Assembly 54, 2001). Questions related to breastfeeding included: if breastfeeding was initiated after birth and when, type of milk fed to the infant, frequency, duration, mode (breast/bottle), and reasons for breast/bottle feeding. A complimentary feeding questionnaire that included questions related to food groups consumed during the previous day and age of introduction of foods and beverages (i.e., water, milk, sugar sweetened beverages, cereals, pulses, vegetables, animal protein, and dairy) was applied.

Breastfeeding in the first 6 months of life was classified according to WHO recommendations as: 1) exclusive, when only breast milk is fed to the infant; 2) predominant, when in addition to breastmilk, the infant is given water, unsweetened tea and other beverages excluding formula milk; 3) partial, when in addition to breastmilk the infant is given formula, solid foods or other milks; and 4) not breastfeeding.

Considering the sample size, and that the most prevalent categories during the first 6 months of life were exclusive and partial breastfeeding, we decided to focus on exclusive breastfeeding duration. The above, considering that predominantly breastfed infants and not breastfed infants were scarce for independent categories. For example, during the first month of life, 147 (55.9%) infants had partial breastfeeding, 106 (40.3%) exclusive, 7 (2.7%) predominant, and 3 (1.1%) had no breastfeeding. At 3 months, 132 (48.9%) infants had partial breastfeeding, 112 (41.5%) exclusive, 16 (5.9%) no breastfeeding, and 10 (3.7%)

predominant breastfeeding. Lastly, at 6 months, most already had partial breastfeeding: 238 (80.4%) infants had partial breastfeeding, 8 (2.7%) exclusive, 4 (1.4%) predominant, and 46 (15.5%) had no breastfeeding. Therefore, exclusive Breastfeeding (EBF) duration patterns were classified as follows: 1) EBF <1 month (reference group that includes those who breastfed exclusively < 1 months, or that had predominant or partial breastfeeding during the first 6 months of life, or had no breastfeeding), 2) 1-3 months of EBF, and 3) >3 to ≤6 months of EBF. Continuous breastfeeding (CBF), from 6 months of age forward was classified as present in cases were mothers reported breast milk consumption and absent otherwise.

2.2.2 Infant growth and adiposity indicators

Weight, length, abdominal circumference, and skinfold thicknesses (tricipital, bicipital, subscapular, and suprailiac) of the child were obtained at birth, 1, 3, 6, 9, 12, 18 and 24 months of age using standardized procedures (Lohman et al., 1988). Personnel were trained and standardized at the beginning of the study to ensure consistent and accurate measurements (Habicht, 1974). Anthropometric measurements at birth were not obtained by our project personnel due to logistic difficulties in 170 cases, which amounted to ~45% of study sample. In those cases, birth weight was obtained from medical records. All measurements were taken twice and averaged, and skinfold thicknesses were measured by triplicate. Children were weighed wearing light clothing with a portable electronic paediatric scale (Tanita BABY MOMMY model 1582) with a precision of 10g. The length was measured using a wood infantometer (Schorr) with a precision of 0.1 cm. Abdominal circumference was measured with a fiberglass tape with a precision of 0.1 cm. Skinfolds were measured with a Lange skinfold calliper to the nearest 1.0 mm, while applying a constant pressure; the mean values of the anthropometric measurements were used in the analyses. We used WHO-Anthro macro for Stata to estimate Z-scores of weight-for-length (ZWFL), body mass index-for-age (ZBMI) and length-for-age (ZLEN), based on World Health Organization (WHO) growth standards (Law et al., 2004).

2.2.3 Appetite and satiety regulation indicators

We applied the Child Eating Behaviour Questionnaire (CEBQ) (Sleddens et al., 2008a; Wardle et al., 2001) to the mothers at months 6, 9, 12, 18 and 24. The CEBQ is a parent-report measure comprised of 35 items, each rated on a five-point Likert scale that ranged from never to always. It is made up of eight subscales: (1) food responsiveness, (2) enjoyment of food (a high score generally reflects the children’s appetite for food or desire to eat in response to food exposure, and these have been associated with obesity); (3) satiety responsiveness (a higher score represents a child's ability to reduce their food intake after eating to regulate energy intake, sensitivity to satiety signals); (4) slowness in eating (high scores "characterize a reduction in the rate of eating as a result of a lack of enjoyment and interest in food"); (5) desire to drink (high score reflects the children's desire to have drinks to take with them, usually sugary drinks); (6) food fussiness (high score refers to the rejection of a substantial amount of familiar and new foods, leading to an inappropriate variety of food intake); (7) emotional overeating and (8) emotional undereating (a high score on these characterizes increased or decreased eating in response to negative emotions) (Carnell & Wardle, 2007a; Sleddens et al., 2008b). CEBQ was selected because it is a standardized subjective measure of infant appetite, designed to characterize appetitive traits that have been associated to excess weight gain (Carnell & Wardle, 2007b), as well as with breastfeeding (Brown & Lee, 2012). CEBQ questionnaire has been previously validated in Mexico in a low sociodemographic sample (Hunot-Alexander et al., 2021). For this study, CEBQ was translated and validated as part of the MAS-Lactancia study. The CEBQ was translated to Spanish and back translated to English (to assess consistency), after evaluating if the instrument was adequately understood, the translated Spanish version of the instrument was validated with psychometric analysis at ages 6, 12, 18, and 24 months (publication in process). Most subscales had acceptable Cronbach's alpha ($\alpha \geq 0.60$ to <0.80). Food responsiveness (12 months), enjoyment of food (12, 18, and 24 months), and food fussiness (24 months) had high internal consistency ($\alpha > 0.80$). However, it is worth noting that some subscales had lower performance ($\alpha \geq 0.50$ to <0.60), such as emotional over-eating (12 and 18 months), satiety responsiveness (6 and 12 months), and slowness in eating (6 and 24 months).

2.3 Covariates

Information about household characteristics, maternal education, occupation, and maternal BMI was measured at recruitment. Also, we calculated the number of counselling visits that the mothers attended from recruitment to 24 months post-partum. A household wealth index (HWI) was generated using principal component analysis, and included housing conditions (housing type, floor, walls, and roofing construction materials), water and sanitation services, ownership of home appliances, electronics, and number of rooms. The methodology to derive the index has been published elsewhere (Gutiérrez, 2013; Kim-Herrera et al., 2021).

2.4 Statistical analysis

For descriptive analysis of categorical variables, we calculated frequencies and percentages, as well as p values with Pearson's chi squared tests. Continuous variables with normal distribution were summarized as means and standard deviations, and those without normal distribution as 50^{th} percentile and interquartile range. For p value calculations, we used Student's t tests and Wilcoxon's rank-sum test accordingly.

We used linear regressions adjusted for maternal education, maternal occupation, maternal civil status, HWI, maternal BMI at recruitment, child birth weight, and child's sex to evaluate the association between EBF and the sub-scales of CEBQ; and used linear mixed models to evaluate the association between patterns of breastfeeding with growth indicators and adiposity (ZBMI, ZWFL, ZLEN, skinfold sum, and abdominal circumference) from 1 month to 24 months of age for EBF and from 6 months through 24 months of age for CBF. Considering that sex-related differences in adipose tissue utilization and storage have been reported in infants (Davis et al., 2019; Gale et al., 2015; Hoffman & Valencak, 2021) and that there are also cultural differences in breastfeeding according to child's sex (Jama et al., 2020; Jayachandran & Kuziemko, 2011), we tested for effect modification by sex by including a product term between child's sex and EBF or CBF exposure in the models.

For ZBMI, ZWFL, and ZLEN, models were adjusted by maternal education, maternal occupation, maternal BMI at recruitment, HWI, child's birthweight, and the number of counselling visits. For abdominal circumference and skinfold sum, models were additionally adjusted by the child's age and sex. For CBF, models were additionally adjusted by EBF. All covariates were selected according to our conceptual framework using directed acyclic diagrams. Finally, to estimate the mediating role of appetite and satiety indicators in the association between breastfeeding patterns with growth and adiposity indicators, we used path analysis with standard error accounting for intragroup correlation at the child level. Models were computed for EBF and CBF separately, and for every CEBQ subscale and growth/adiposity outcome independently (using longitudinal information of CEBQ subscales and growth/adiposity indicators). We adjusted the false discovery rate for mediation analysis using three scenarios (false discovery rate of 5%, 10% and 20%) with the Benjamini-Hochberg procedure.

According to the American Statistical Association statement (Wasserstein & Lazar, 2016), which calls for scientific conclusions to not be based on whether a p-value passes a specific threshold, we interpreted our results considering that smaller p-values together with the value of the estimates provide more evidence against the nullity of the association under the specified model. All statistical analyses were performed using Stata V.14 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX, USA: StataCorp LP.).

3. Results

3.1 Characteristics of the study population

The analysed sample differed from the sample not included in the analytical sample because of incomplete information (n = 385) in three characteristics. Children in the analytical sample had a higher proportion of longer duration of CBF (12-17.9 months and ≥18 months) than not included children. Likewise, not included children had lower proportion of mothers with education level of high school or more (25.4%) and basic or less (24.85%), and their mothers attended fewer counselling visits than mothers in the analytical sample (3.6 vs. 6.2 visits). (**Supplemental Table 1**).

Characteristics of the mother and children in the study sample (N=378) are presented in **Table 1**. Participant's mothers had a mean BMI at recruitment of 26.3 Kg/m^2, 32% had an education level of high school or more, around half had a formal employment, and the average counselling visits received were of 6.2 visits. Half of the children were females, and 43% had >3 to ≤6 months of EBF duration. Around 44% had continued breastfeeding at least until 12 months of age. No continued breastfeeding (NBF) was observed in 19.4 % of study participants. Most children´s birth weight was normal (≥2500 - <4000 g).

The association between patterns of breastfeeding and appetite and satiety indicators are shown in **Supplemental Table 2**. We found that longer duration of EBF (>3 to ≤6m EFB vs ref.) was associated with a lower CEBQ score in the enjoyment of food scale at 9 (β= -1.11, 95%CI -1.92, -0.29, P= 0.01), 12 (β= -1.63, 95%CI -2.58, -0.68, P<0.01), 18 (β= -0.99, 95%CI -2.08, 0.09, P= 0.07), and 24 months (β= -1.09, 95%CI -2.31, 0.12, P= 0.08). Longer EBF was also associated with a higher score in the satiety responsiveness scale at 6 months (1-3 months EBF vs. ref β= 1.31, 95%CI 0.04, 2.58, P= 0.04), and at 9 to 12 months (EBF > 3 months vs. ref: 9 months β= 1.14, 95%CI 0.07, 2.21 P= 0.04; 12 months β= 0.88, 95%CI -0.08, 1.85, P= 0.07). Also, >3 to ≤6 months of EBF was associated with a higher score in slowness in eating construct at 9 (β=1.27, 95%CI 0.19, 2.35, P=0.02) and 18 months (β=1.00, 95%CI -0.12, 2.13, P= 0.08), and with higher scores in food fussiness scale at 9 (β=2.31, 95%CI 0.98, 3.62, P<0.01) and 12 months (β=2.57, 95%CI 1.17, 3.98, P<0.01).

Finally, receiving EBF from 1 to 3 months was associated with less food responsiveness scale at 12 months (β= -2.07, 95%CI -4.17, -0.03, P= 0.05).

3.2 Association between breastfeeding patterns with adiposity and growth indicators

The association between breastfeeding patterns with growth and adiposity indicators is summarized in **Table 2**. In relation to EBF, we found effect modification in terms of sex with abdominal circumference, ZWFL, and ZLEN. Compared to the reference group (EBF <1 month), boys with >3 to ≤6 months of EBF had 0.66 cm less abdominal circumference (β= -0.66; 95%CI -1.32, 0.00; p=0.05). In girls, this relation was positive. In ZWFL a similar relation was observed, with boys with >3 to ≤6 months of EBF having lower Z scores than the reference group (β= -0.17; 95%CI -0.42, 0.08; p=0.19) and girls having a relatively small positive effect size. Regarding ZLEN, compared to the reference group, boys with EBF >3 to ≤6 months and those with 1-3 months of EBF had lower Z scores than the reference group (β= -0.49; 95%CI -0.73, -0.25; p<0.01 and β= -0.37; 95%CI -0.67, -0.08; p=0.01, respectively). Girls with >3 to ≤6 months of EBF had also lower Z scores than the reference group, and those with 1-3 months had relatively higher Z scores. No effect modification by sex was detected for ZBMI and skinfold sum. A relatively small positive relation was identified between EBF and ZBMI. Regarding skinfold sum, a positive relation with EBF categories was also observed.

As for CBF, no effect modification by sex was identified for any outcome. Infants without CBF had higher ZBMI (β= 0.19; 95%CI 0.08, 0.30; p<0.01) and abdominal circumference (β= 0.62; 95%CI 0.19, 1.05; p=0.01), as well as lower skinfold sum (β= -0.80 95%CI -1.72, 0.13; p=0.09) than infants with CBF. Also, infants without CBF had a relatively higher ZWFL and lower ZLEN.

3.3 Mediating role of appetite and satiety indicators

Estimates of the mediating role of appetite and satiety indicators in the association between EBF and CBF with growth and adiposity were summarized in **Table 3**. Most of the total effect of EBF and CBF in adiposity and growth indicators is a direct effect. With respect to the exposure of having >3 to ≤6 months of EBF, the indirect effect through satiety responsiveness accounted for around 14% (β= -0.02, 95%CI -0.04, 0.00, P= 0.10) of the total effect on ZBMI (β= -0.14, 95%CI -0.36, 0.09, P= 0.24). Also, the indirect effect through food fussiness (β= -0.07, 95%CI -0.14, 0.01, P= 0.08) represented around 11% of the total effect on abdominal circumference (β= -0.65, 95%CI -1.20, -0.10, P= 0.02) and ~7% (β= -

0.03, 95%CI -0.06, -0.00, P= 0.04) of the total effect on ZLEN (β= -0.43, 95%CI -0.63, -0.23, P= 0.000). Regarding food enjoyment, the indirect effect represented around 5% (β= -0.02, 95%CI -0.04, 0.00, P= 0.10) of the total effect on ZLEN (β= -0.44, 95%CI -0.63, -0.24, P= 0.000). As for the exposure of having CBF, the indirect effect for food fussiness accounted for ~50% (β= -0.02, 95%CI -0.05, 0.00, P= 0.09) of the total effect on ZLEN (β= -0.04, 95%CI -0.21, 0.13, P= 0.64). Additional information on all calculated estimates can be found in Supplementary Material (**Supplemental Table 3**). False discovery rate and P value adjustment using the Benjamini-Hochberg procedure in Supplemental Table 3 can be found online at Figshare: https://doi.org/10.6084/m9.figshare.23627973. When using this adjustment, indirect effects are no longer conventionally statistically significant.

4. Discussion

Results of this study suggest that breastfeeding patterns are associated with changes in adiposity and growth in children under 2 years of age, and that this association is partially mediated by appetite and satiety indicators such as satiety responsiveness, food fussiness and food enjoyment. For EBF, we found evidence of effect modification by sex. Longer exposure to EBF resulted in lower abdominal circumference, weight-for-length, and length-for-age Z scores in boys, while in girls point associations were positive. No effect modification was found for ZBMI and skinfold sum. Point estimates for skinfold sum and longer exposure to EBF were positive. For CBF, no effect modification was found, and our results suggest that independently of EBF, CBF has a positive association with adiposity in children. Lower exposure to CBF was associated with higher ZBMI and abdominal circumference, and lower skinfold sum. Also, our estimates suggest that around 5-14% of the total effect of breastfeeding on adiposity and growth could be mediated through appetite and satiety indicators such as satiety responsiveness, food fussiness and food enjoyment. However, mediation results should be taken cautiously since effects were observed only for the above subscales (which are subjective measures) and some outcomes (abdominal circumference, length-for-age Z score, and BMI for age Z score).

Taken together, these results suggest that there may be a difference in adipose tissue storage according to EBF exposure and sex, as well as by CBF exposure. Previous studies have reported that breastfed infants tend to have more adipose tissue than formula-fed infants in the first 6 months of life (Butte et al., 2000; Gale et al., 2012; Tahir et al., 2021). However, this observation could be partly due to different adipose tissue distributions. In our study, we found evidence of higher skinfold sum in infants of both sexes with >3 to ≤6 months of EBF, but lower abdominal circumference in boys. Also, those without CBF had higher ZBMI score and abdominal circumference, but lower skinfold sum from 6 to 24 months of life. Considering the importance of abdominal fat accumulation, which has been documented to result in higher risk than peripheral accumulation (World Health Organization, 2000) and is considered a proxy of visceral fat, these differential distributions could partly explain the lower cardiovascular risk in future life stages (Martin et al., 2005; Parikh et al., 2009; Rich-Edwards et al., 2004).

It is noteworthy that although EBF (0 to 6 months) was associated to a lower length-for-age, CBF from 6 to 24 months was not, which could be due to different growth patterns between breastfed children and formula-fed children previously shown in the literature (Dewey, 1998a, 1998b). However, the evidence behind the effect that breastfeeding has on growth is uncertain, given that some studies have concluded that breastfed children have lower growth (Jia et al., 2018; Zong et al., 2020), others that they have a greater growth (Martin, 2002; Wallenborn et al., 2021), or that the difference depended on the age analyzed (Agostoni et al., 1999). In this regard, a recent systematic review documented that longer duration of exclusive and partial breastfeeding tend to be associated with slower growth during infancy only in the developed settings (Bernadeta et al., 2019), which could partly explain this observation and the controversy between studies.

Regarding appetite and satiety indicators, our results suggest that longer EBF and CBF durations are associated with lower food enjoyment, and higher satiety responsiveness, higher food fussiness and slowness in eating. Additionally, CBF duration was associated with higher scores in emotional under-eating and desire to drink. These results are similar to those of Masztalerz-Kozubek *et al.* (Masztalerz-Kozubek et al., 2022), who found that longer breastfeeding duration in Polish infants was negatively related to food enjoyment and positively related to satiety responsiveness and slowness in eating subscales. However, results are contradictory with other studies with respect to desire to drink and food fussiness subscales. For desire to drink, Yelverton *et al.* (Yelverton et al., 2021) reported that non-breastfed Irish children had higher possibilities of expressing desire to drink, and Masztalerz-Kozubek *et al.* (Masztalerz-Kozubek et al., 2022) concluded that longer duration of breastfeeding was negatively associated to this construct. For food fussiness, Pang *et al.* (Pang et al., 2020) reported that longer breastfeeding duration was associated with lower levels of food fussiness at 3 years of age in Singapore. Differences in these results could be due to cultural differences in the understanding of these constructs. Specially, since some subscales do not specify what is being referenced. For example, desire to drink scores could be high due to water or sugar sweetened beverage desire, which have different metabolic implications. However, analyzing the possibility of mediation of these constructs in the association between breastfeeding with adiposity and growth indicators, we only found evidence of small effect size for satiety responsiveness, food fussiness and food enjoyment. Small effect sizes could be due to the fact that appetite and satiety was measured subjectively through parent report. Therefore, it is possible that we may be underestimating the true effect.

The main strength of our study relies on its longitudinal design, the richness and quality of the information obtained from the follow-up of participants, such as detailed breastfeeding patterns during the 24 first months of life, appetite and satiety indicators, and adiposity measurements at several stages, as well as the longitudinal approach for association analysis and the inclusion of relevant covariates. Our study also has limitations that should be considered. Reverse causality in the association between breastfeeding, appetitive traits, and adiposity/growth indicators, although unlikely due to the temporality of the variables and adjustment by birthweight and maternal BMI in the third trimester (proxy to appetitive traits at baseline), cannot be ruled out entirely as have others previously pointed out (Kramer et al., 2011), so results should be taken with caution. As any cohort study, attrition rate during follow-up reduced the sample size to ~50% of the original sample. This resulted in differences between included and excluded samples, such as a longer duration in continued breastfeeding, higher education, and counselling visits in the included sample. However, all analysis were adjusted by the mother’s education level and counselling visits attended. Regarding associations evaluated in EBF and CBF duration models, we argue that we may be underestimating the point estimates obtained, since the reference category has a relatively healthier breastfeeding pattern. The above, considering that since this cohort included an intervention for breastfeeding promotion, the no breastfeeding group was considerably reduced. This could also have contributed to lack of differences or statistical significance in some estimations. Also, the CEBQ is a validated instrument to study children´s behaviours related to appetite and satiety mechanisms; however, studies that have applied it in young children are scarce, so we consider that more research is required on the subject together with the exploration of other quantitative indicators of appetite and satiety, and/or metabolism of adipose tissue. Also, it is worth noting that satiety responsiveness subscale had low internal consistency at 6 and 12 months of age. However, since we considered all items from this subscale were relevant and that Cronbach's alpha improved with age (α= 0.64 and α= 0.70 at 18 and 24 months, respectively), we decided to keep the original subscale structure. A possible explanation to the above could be related to the fact that parents are just beginning complementary feeding with their infants and are not yet highly trained/sensitive in this type of food cues. Specially, considering that internal consistency of the satiety responsiveness subscale seems to improve with age.

Finally, even though there was effect modification by sex in EBF models, we could not estimate mediation analysis considering effect modification due to sample size restrictions.

In conclusion, our results suggest that longer breastfeeding exposure is associated with less adiposity and more favourable adipose tissue distributions, and that growth indicators (length-for-age) may only be affected during the first six months of life, since EBF was associated with lower length-for-age, but CBF (adjusting by EBF) was not, all of which seems to be partly mediated through appetite and satiety indicators. However, it is important to consider that reverse causality cannot be completely discarded. More studies are necessary to understand how appetite and satiety mechanisms are involved in appetite and satiety indicators and the development of obesity and other metabolic diseases in further stages of life.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

JRD and IRS were responsible for the conception of the cohort study and the current analysis. JRD, IRS, ACA, EZ, and LA were responsible for the design of the cohort study. JRD as well as IRS, and ACA obtained funding for initial and later follow ups, respectively. IRS, SB, ACA, EOP and MM were responsible for acquisition and analysis of data. IRS, SB, ACA and JRD were responsible for drafting the manuscript. All authors (IRS, SB, EOP, SB, ACA, JRD, MMJ, ABG, EZ, LA) critically revised the manuscript for important intellectual content, approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Ethical statement

The study was conducted according to principles described in the Declaration of Helsinki. This study (project 1281) was approved by the Human Research and Ethics Committees at the National Institute of Public Health and IMSS in Mexico (approval number 1646). Written informed consent were obtained from all women at each stage of the study.

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Declaration of competing interest

The authors have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

Data is not able to be made openly available because this is an ongoing birth cohort in which recruitment is still active. However, the particular data set used for analysis that led to the manuscript can be made available via a request to the corresponding author (JR). A formal data sharing agreement will be required in which the conditions for the use of this data for publication purposes will be stated.

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Figure 1.- Study sample from Mas-Lactancia birth cohort

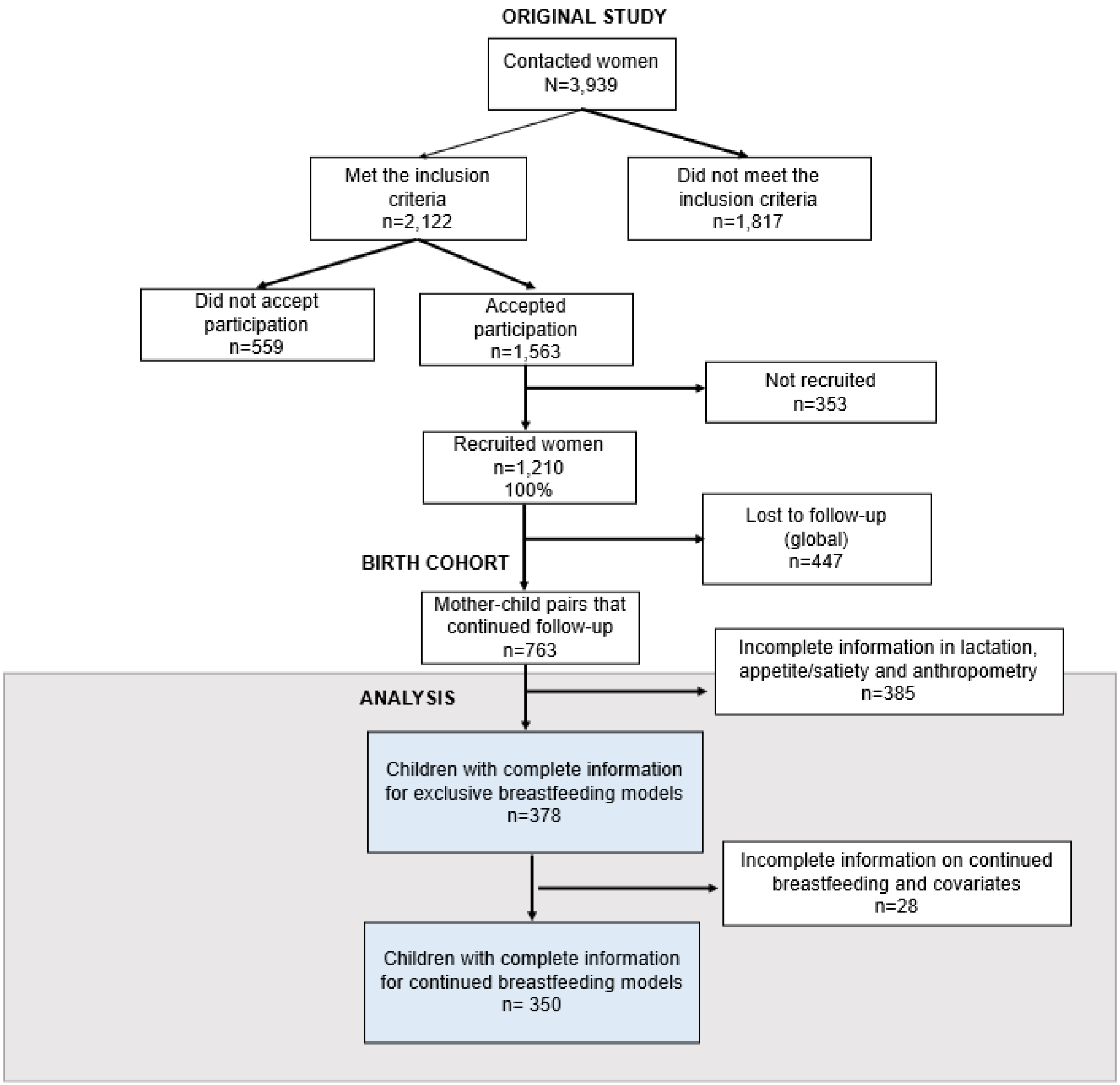

Table 1. General characteristics of the MAS-Lactancia birth cohort population studied.

General characteristics	n	Study population N=378 Value
<u>Mother</u>		
Body Mass Index (Kg/m^2), μ ± S.D.	378	26.3 ± 4.1
Educational level, %		
Basic or less	107	28.3
Secondary	150	39.7
High school or more	121	32.0
Occupation, %		
Home	126	33.3
Informal or student	54	14.3
Formal	198	52.4
Household wealth index, %		
Low	122	32.4
Medium	130	34.4
High	126	33.3
Counselling (n° visits), μ ± S.D.	378	6.2 ± 2.4
<u>Child</u>		
Sex,		
% Female	185	48.9
Exclusive breastfeeding, %		
<1 month, other breastfeeding modalities, or no breastfeeding	147	38.9
1 – 3 months	70	18.5
>3 to ≤6 months	161	42.6
Continued breastfeeding, %		
No continued breastfeeding	68	19.4
6 – 8.9 months	88	25.1
9 – 11.9 months	40	11.4
12 – 17.9 months	68	19.4
≥18 months	86	24.6
Missing, n	28	-
Birth weight, %		
Low (<2500 g)	18	4.8
Normal (≥2500 - <4000 g)	354	93.6
Macrosomia (≥4000 g)	6	1.6
Body Mass Index Z score, μ ± S.D.		
6 months	297	0.1 ± 1.0
12 months	243	0.1 ± 1.0
24 months	141	0.1 ± 1.0
Weight-to-length Z score, μ ± S.D.		
6 months	297	0.2 ± 1.0
12 months	243	-0.1 ± 1.0
24 months	141	0.0 ± 0.9
Abdominal circumference (cm), μ ± S.D.		
6 months	296	42.5 ± 2.6
12 months	244	43.8 ± 3.1
24 months	136	46.0 ± 3.0
Skinfold sum (mm) , μ ± S.D.		
6 months	297	28.8 ± 6.1
12 months	244	26.6 ± 6.3
24 months	136	23.3 ± 6.3
Length-for-age Z score, μ ± S.D.		
6 months	297	-0.8 ± 0.9
12 months	245	-1.0 ± 1.1
24 months	143	-0.9 ± 1.0

Table 2. Association between exclusive and continued breastfeeding with adiposity and growth indicators from 1 to 24 months by sex.

	Female			Male			Total		
Adiposity/growth indicators	β	IC95%	P value	β	IC95%	P value	β	IC95%	P value
Exclusive breastfeeding duration [1]									
Body Mass Index Z score									
<1 month EBF, other breastfeeding modalities, or no breastfeeding	--	--		--	--		*Ref.*	--	
1-3 months EBF	--	--		--	--		0.09	-0.14, 0.32	0.45
>3 to ≤6 months EBF	--	--		--	--		0.03	-0.15, 0.22	0.72
Weight-for-length Z score									
<1 month EBF, other breastfeeding modalities, or no breastfeeding	*Ref.*	--		*Ref.*	--		--	--	
1-3 months EBF	0.09	-0.25, 0.43	0.62	0.03	-0.28, 0.35	0.83	--	--	
>3 to ≤6 months EBF	0.09	-0.17, 0.35	0.48	-0.17	-0.42, 0.08	0.19	--	--	
Abdominal circumference (cm)									
<1 month EBF, other breastfeeding modalities, or no breastfeeding	*Ref.*	--		*Ref.*	--		--	--	
1-3 months EBF	0.46	-0.45, 1.36	0.32	-0.14	-0.96, 0.68	0.74	--	--	
>3 to ≤6 months EBF	0.41	-0.27, 1.09	0.24	-0.66	-1.32, 0.0	0.05	--	--	
Skinfold sum (mm)									
<1 month EBF, other breastfeeding modalities, or no breastfeeding	--	--		--	--		*Ref.*		
1-3 months EBF	--	--		--	--		0.31	-1.02, 1.63	0.65
>3 to ≤6 months EBF	--	--		--	--		0.58	-0.46, 1.62	0.28
Length-for-age Z score									
<1 month EBF, other breastfeeding modalities, or no breastfeeding	*Ref.*	--		*Ref.*	--		--	--	
1-3 months EBF	0.19	-0.10, 0.47	0.24	-0.37	-0.67, -0.08	0.01	--	--	
>3 to ≤6 months EBF	-0.18	-0.35, 0.09	0.16	-0.49	-0.73, -0.25	0.000	--	--	
Presence of continued breastfeeding [2]									
Body Mass Index Z score									
No CBF vs. CBF	--	--		--	--		0.19	0.08, 0.30	0.001
Weight-for-length Z score									
No CBF vs. CBF	--	--		--	--		0.05	-0.06, 0.16	0.36
Abdominal circumference (cm)									
No CBF vs. CBF	--	--		--	--		0.62	0.19, 1.05	0.01
Skinfold sum (mm)									
No CBF vs. CBF	--	--		--	--		-0.80	-1.72, 0.13	0.09
Length-for-age Z score									
No CBF vs. CBF	--	--		--	--		-0.02	- 0.12, 0.08	0.67

EBF: Exclusive Breastfeeding; CBF: Continued Breastfeeding

Reference group for EBF models are infants without breastfeeding/other breastfeeding modalities/<1month of EBF.

1. Mixed models adjusted by: maternal education, maternal occupation, maternal BMI at recruitment, household wealth index, child's birthweight, and the number of counselling visits, for ZBMI, ZWFL, and ZLEN. Abdominal circumference and Skinfold sum models were additionally adjusted by the child's age and sex.

2. Mixed models, adjustment covariates similar to EBF models (1), with the addition of exclusive breastfeeding.

Table 3. Mediating role of appetite and satiety indicators in the association between exclusive breastfeeding duration and presence of continued breastfeeding with growth and adiposity indicators

	Path type in path analysis models of growth and adiposity indicators								
	Direct			**Indirect**			**Total**		
Adiposity indicator/age period	β	(IC95%)	**P value**	β	(IC95%)	**P value**	β	(IC95%)	**P value**
Exclusive breastfeeding duration[1]									
Body Mass Index Z score									
<1 month EBF, other breastfeeding modalities, or no breastfeeding	*Ref.*	--		*Ref.*	--		*Ref.*	--	
1-3 months EBF									
Satiety responsiveness	0.04	-0.23, 0.30	0.79	-0.01	-0.03, 0.01	0.46	0.03	-0.24, 0.30	0.83
>3 to ≤6 months EBF									
Satiety responsiveness	-0.12	-0.34, 0.10	0.29	-0.02	-0.04, 0.00	0.10	-0.14	-0.36, 0.09	0.24
Abdominal circumference (cm)									
<1 month EBF, other breastfeeding modalities, or no breastfeeding	*Ref.*	--		*Ref.*	--		*Ref.*	--	
1-3 months EBF									
Food fussiness	-0.19	-0.95, 0.58	0.63	0.02	-0.04, 0.07	0.52	-0.17	-0.94, 0.60	0.67
>3 to ≤6 months EBF									
Food fussiness	-0.59	-1.15, -0.03	0.04	-0.07	-0.14, 0.01	0.08	-0.65	-1.20, -0.10	0.02
Length-for-age Z score									
<1 month EBF, other breastfeeding modalities, or no breastfeeding	*Ref.*	--		*Ref.*	--		*Ref.*	--	
1-3 months EBF									
Food fussiness	-0.20	-0.51, 0.11	0.20	0.01	-0.02, 0.03	0.59	-0.19	-0.51, 0.12	0.22
Food enjoyment	-0.20	-0.51, 0.11	0.20	0.00	-0.01, 0.02	0.61	-0.20	-0.51, 0.11	0.21
>3 to ≤6 months EBF									
Food fussiness	-0.40	-0.60, -0.20	0.000	-0.03	-0.06, -0.00	0.04	-0.43	-0.63, -0.23	0.000
Food enjoyment	-0.42	-0.62, -0.22	0.000	-0.02	-0.04, 0.00	0.10	-0.44	-0.63, -0.24	0.000
Presence of continued breastfeeding[2]									
Length-for-age Z score									
No CBF	*Ref.*	--		*Ref.*	--		*Ref.*	--	
CBF									
Food fussiness	-0.02	-0.19, 0.15	0.82	-0.02	-0.05 – 0.00	0.09	-0.04	-0.21, 0.13	0.64

EBF: Exclusive Breastfeeding; CBF: Continued Breastfeeding

1.Path models adjusted by: maternal education, maternal occupation, maternal BMI at recruitment, household wealth index, child's birthweight, and the number of counselling visits for ZBMI, ZWFL, and ZLEN. Abdominal circumference and Skinfold sum model was additionally adjusted by the child's age and sex.

2.Path models with adjustment covariates similar to EBF models (1), and additionally adjusted by duration of exclusive breastfeeding