Association of Exclusive Breastfeeding Duration with Systemic Inflammation Markers in Adolescents: A Cross-Sectional Study

Running title: Breastfeeding and systemic inflammation

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Abstract

Background: Breastfeeding duration has been associated with less low-grade inflammation

in healthy adolescents, but there is scarce information regarding obese subjects. This study aimed to evaluate whether exclusive breastfeeding is related to serum concentrations of inflammatory markers in a population of Spanish adolescents.

Methods: A cross-sectional study was performed on 1001 adolescents (13.2±1.2 years) randomly recruited from schools in southeast Spain. Data on breastfeeding duration were collected via a parental questionnaire. Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) were determined by ELISA. C-reactive protein (CRP) was determined by solid-phase chemiluminescent immunometric assay.

Results: Nonadjusted and adjusted multivariate correlation analyses confirmed a strong association ($p < .001$, CI 95%) between the 3 markers of inflammation and exclusive breastfeeding duration. No significant differences were observed for IL-6, TNF-α, and CRP serum concentrations among normal-weight, overweight and obese adolescents, except for IL-6 between normal-weight and obese subjects. Likewise, no significant association was found between these markers of inflammation and body mass index z-score.

Conclusions: We found a possible association between inflammatory markers and exclusive breastfeeding duration in adolescents, regardless of their body mass index. This finding suggests that increased body weight or obesity might not mediate the association between breastfeeding and inflammation. These results contribute to the understanding of the relationship between breastfeeding and inflammatory markers in adolescents.

Keywords: adolescents, breastfeeding, inflammation, obesity.

The World Health Organization and major scientific societies, such as the American Academy of Pediatrics, consider breastmilk to be the ideal food for ensuring proper growth and development of children (American Academy of Pediatrics Section on Breastfeeding, 2005; World Health Organization & UNICEF, 2003). González-Jiménez Garcia, Aguilar, Padilla, and Alvarez (2014) and Horta, Bahl, Martines, Victora, and Horta (2007) have shown the positive effects of breastfeeding on the health of both the mother and the child in their reviews of the literature. In a previous study, Labayen et al. (2012) demonstrated one of the positive effects when they found that exclusive breastfeeding, (i.e., maternal milk as the only form of nourishment) for more than 6 months was associated with a decrease in low-grade inflammation, as estimated by serum fibrinogen levels, in healthy children and adolescents. The role of breastfeeding in modulating low-grade inflammation in obese subjects, however, remains unknown. This low-grade inflammation is expressed as changes in the serum levels of certain inflammatory markers, such as interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and C-reactive protein (CRP), which are associated with the early development of cardiovascular disorders in obese subjects (González-Jiménez, Schmidt-Riovalle, Sinausía, Carmen Valenza, & Perona, 2016).

Breastfeeding is associated with a decreased incidence of obesity and contributes to improving the state of chronic low-grade inflammation in obese subjects (Das, 2001). Nevertheless, evidence regarding related factors that might influence obesity-related inflammation, such as duration of breastfeeding, has been mixed (Labayen et al., 2012; Singhal, Cole, Fewtrell, & Lucas, 2004; Vérier et al., 2011) and has been primarily focused, in most cases, on very specific populations. For instance, Singhal et al. (2004) found a significantly lower CRP concentration in 13−16-year-old British adolescents who had been breastfed as preterm infants than in those who had been fed a preterm formula.

In contrast, Vérier et al. (2011) observed only marginal associations between

breastfeeding duration and CRP levels in healthy adolescents after adjustment for cardiovascular risk factors. In this context, and given that breastfeeding is associated with reductions in obesity prevalence later in life, the aim of the present study was to evaluate whether breastfeeding duration is related to serum concentrations of markers of low-grade inflammation in a population of Spanish adolescents.

Methods

Design and Participants

The present cross-sectional study included 1001 adolescents with a mean age of 13.2 ±1.2 years. We conducted the study from October 2013 to June 2014, and adolescents from 18 high schools in the provinces of Granada and Almeria in southeast Spain participated. We selected schools based on the demographic characteristics in both provinces using data from the National Institute of Statistics, which ensured that we achieved a representative sample in both provinces. We submitted an invitation letter to the principals of the selected schools, which contained an information sheet and a presentation with the study details. Among the 18 selected schools, we and the principals randomly invited students in two classes per grade to participate in the study. An important criterion for inclusion in the study was that participants had to be healthy and not have any type of endocrine dysfunction, physical disorder or infectious process of any kind. Among the 2160 potentially eligible students in the selected classes, we examined 1200 for eligibility and recruited 1001. All participants completed the study.

Anthropometric Measurements, Blood Pressure and Breastfeeding Duration

We also interviewed the 1200 potential participants and their parents to determine whether or not they met the inclusion criteria. Participants then underwent a complete anthropometric evaluation to ascertain their nutritional status. We performed this evaluation according to the recommendations of the European Pediatric Association (body composition

analysis protocol, Claessens, Beunen, & Malina, 2000). The variables we assessed were weight, height, body mass index (BMI), waist and hip circumferences, waist-to-hip ratio (WHR) and triceps, biceps, subscapular, and suprailiac skinfolds. A trained member of the research team collected anthropometric measures and blood pressure in a classroom enabled for this purpose by the principal of each school. We weighed the students on a self-calibrating Seca® 861 class (III) digital floor scale, which has a precision up to 100 g. We measured their height with a Seca® 214* portable stadiometer using the following procedure: We asked students to stand upright with back, buttocks, and heels against the stadiometer and head oriented in the Frankfurt plane before placing the horizontal headpiece on the top of their head to measure height. We defined overweight and obesity according to the international standards that Cole, Bellizzi, Flegal, & Dietz (2000) established, as BMI values appropriate for age and sex that were above the 85th and 95th percentiles, respectively. We measured waist circumference using the horizontal plane midway between the lowest rib and the upper border of the iliac crest at the end of normal inspiration/expiration and hip circumference at the maximum extension of the buttocks as viewed from the right side. For both measurements, we used a Seca® automatic roll-up measuring tape (precision of 1 mm) to measure students who were in a standing position with their arms hanging at their sides in a normal anatomical position. We calculated the WHR by dividing the waist circumference by the hip circumference. We measured triceps, biceps, subscapular, and suprailiac skinfolds with a Holtain® skinfold caliper, which has a precision of 0.1−0.2 mm. We used these skinfold measurements along with body density calculated using Brook's equation (Brook, 1971) to calculate the percentage of body fat using the Siri equation (Siri, 1961).

We determined blood pressure levels using a previously calibrated aneroid sphygmomanometer and a Littmann® stethoscope, following the recommendations for blood pressure measurement of the Subcommittee of Professional and Public Education of the

American Heart Association Council on High Blood Pressure Research (Pickering et al., 2005). We interpreted the results based on Korotkoff Phase I for the systolic blood pressure and Phase V for the diastolic blood pressure.

We collected data on breastfeeding duration (in months) via a parental questionnaire that the research team had designed and validated (González Jiménez, 2010). We defined exclusive breastfeeding as an infant's consumption of human milk with no supplementation of any type (no water, no juice, no nonhuman milk and no foods) except for vitamins, minerals and medications. A research team member presented the questionnaire, designed specifically to collect data regarding the duration of breastfeeding, to the mothers in a meeting held in every participating school. The mothers received assistance on completing the questionnaire, and a team member was available to answer their questions.

Serum Biochemical Examination

At 8:00 a.m., in the same classroom in which we had collected anthropometric and blood pressure measures and after students had completed a 12-hr overnight fast, a nurse member of the research team extracted 10 ml of blood by venipuncture in the antecubital fossa of the right arm with a disposable vacuum blood collection tube. Within 4 hr after the extraction, we centrifuged the samples at 1500 rpm for 15 min (Z400 K, Hermle, Wehingen, Germany) to separate the red blood cells. We froze the serum at -80°C for subsequent analysis. Immediately after collection and before centrifugation, however, we measured glucose concentration using an enzymatic colorimetric method (glucose oxidase-phenolaminophenazone [GOD-PAP] method, Human Diagnostics, Germany). We determined serum insulin by radioimmunoanalysis (Insulin Kit, DPC, Los Angeles, CA) and quantified insulin resistance (IR) using homeostasis model assessment–insulin resistance (HOMA-IR; Matthews et al., 1985) by applying the following formula: fasting glucose (mmol/L) x fasting insulin (mU/L)/22.5. We measured IL-6 and TNF-α using enzyme-linked immunosorbent

assay (ELISA; Terumpo Corp., Newark, NJ, and R&D Systems, Minneapolis, MN, respectively). Finally, we measured CRP via high-sensitivity solid-phase chemiluminescent immunometric assay and transformed values to log CRP.

Ethical Issues

We performed this research in strict compliance with the international code of medical ethics established by the World Medical Association and the Declaration of Helsinki. The Board of Education of the Andalusian Regional Government (Granada and Almeria Delegations) approved this study, the school principals authorized it. At the beginning of the study, we obtained written informed consent for students' participation from their parents or guardians, whom we thoroughly informed about study objectives and protocols. A member of the research team (EG-J) recorded and secured the informed consent forms.

Statistical Analysis

We express continuous variables as mean and SD and categorical variables as percentages. To compare BMI across different ages and both sexes, we transformed it to BMI z-score. We log-transformed TNF-α, IL-6 and CRP to normal variables, with significance values of $p < .001$ for the normality test, which we assessed using Kolmogorov-Smirnov's test. To test the relationships between weight classifications (normal weight, overweight and obesity) and inflammatory markers we used ANOVA with post-hoc Tukey's test, whereas to compare the relationships between these markers and breastfeeding for ≤ 6 months or for > 6 months we used Student's t-test. Because of the large number of variables that are potentially associated with inflammation, we carried out a factor analysis to group the variables into common factors that might act as confounding factors. We used varimax rotation with Kaiser normalization to establish relationships among these variables, breastfeeding duration and BMI. Factor analysis involved two procedures: factor extraction to estimate the number of factors and factor rotation to determine the constituents of each factor in terms of the original

variables. We conducted factor extraction using the principal components method. Once we established the number of factors, we conducted factor rotation to determine the composition of factors and calculated factor loadings, which represented correlations of each factor with the original variables. We considered variables that had a factor loading > 0.7 (or < −0.7), that is, variables that showed a strong association with a particular factor, to be major constituents of that factor. We named factors after the group of variables that were its major constituents. We performed multivariate linear regression analysis of breastfeeding duration and inflammatory markers and BMI, adjusting by variables that presented factor loading values > 0.7 (sex, age, body fat, weight at birth, fasting glucose, fasting insulin, waist circumference, BMI z-score, smoking mother and bicipital, tricipital, suprascapular and suprailiac skinfolds). We considered differences to be significant at $p < .05$. To analyze data, we used SPSS software for Linux version 23.0 (IBM Corp, Armonk, NY).

Results

Anthropometric Characteristics of Participants

Participants comprised 1,001 adolescents with an average age of 13.2 ± 1.2 years, 52.1% of whom were female (Table 1). Of the total, 832 adolescents had BMIs within the normal range, while 101 were overweight and 46 were obese and 12.9% had metabolic syndrome. Most participants had been exclusively breastfed for more than 9 months (76.9%), but 3.8% had not been breastfed at all. Mothers of 21.4% of participants smoked during their pregnancy.

Table 1 near here

Factor Analysis

We analyzed TNF-α, IL-6 and CRP to determine the influence of breastfeeding duration and BMI on the systemic-inflammatory status of adolescents. From the factorial analysis (see supplementary table), we extracted two components, one including variables

related to body weight or BMI (body fat, fasting insulin, waist circumference, tricipital skinfold, bicipital skinfold, suprailiac skinfold, suprascapular skinfold, BMI z-score) and the other including variables related to breastfeeding (fasting glucose, birth weight, maternal smoking and breastfeeding duration). The variables listed showed stronger associations (factor loading > 0.7) with the two components extracted. Variables related to Component 1, named Breastfeeding (grouped along y-axis in Figure 1), for which more than 70% of the variance was explained by that single component, demonstrated the strongest influence with TNF-α, IL-6 and CRP. Therefore, the factor analysis showed that these inflammatory markers are strongly associated with the variables related to breastfeeding.

Figure 1 near here

Serum Concentrations of Inflammatory Markers According to BMI and Breastfeeding Groups

Table 2 depicts the serum concentrations of inflammatory markers in normal-weight, overweight and obese adolescents. We observed no significant differences for TNF-α (p = .051) or CRP (p = .058) serum concentrations among the three groups. In the case of IL-6 concentrations, however, the difference between normal-weight and obese adolescents was significant.

Table 2 near here

Table 3 shows the serum concentrations of inflammatory markers in adolescents that were breastfed for ≤ 6 months or > 6 months in their infancy. Differences between groups were very strong for all the three of the markers analyzed (p < .001), with the greatest difference observed for CRP, whose concentration was 4.7 times higher in those breastfed for ≤ 6 months than for those breastfed for < 6 months.

Table 3 near here

Associations Between Inflammatory Markers, BMI and Breastfeeding Duration by

Multivariate Regression Analysis

Table 4 shows statistical parameters for multivariate correlation analyses, before and after adjusting for different confounders. Selected confounders were those variables that presented loading factors > 0.7 (or < −0.7) in the factor analysis. We observed a lack of association between TNF-α, IL-6 and CRP serum concentrations and BMI in both adjusted and unadjusted models. However, we observed clear inverse associations between TNF-α, IL-6 and CRP serum concentrations and exclusive breastfeeding duration up to 13 months, as depicted in Figure 2.

Statistical parameters for nonadjusted and adjusted multivariate correlation analyses confirmed that the associations between serum concentrations of the three inflammatory markers and breastfeeding duration were extremely strong ($p < .001$; Table 4). However, the adjusted model showed that for IL-6 the association was significant (.010) but not as strong as those found for TNF-α and CRP ($p < .001$). It is noteworthy that serum CRP values were approximately double in participants who had been breastfed for less than 4 months compared to those breastfed for more than 4 months (Figure 2).

Figure 2 and Table 4 near here

Since breastfeeding has been associated with obesity and overweight in adolescents, we decided to explore the relationship between these two variables in the present group of adolescents. However, we found no significant association between BMI and exclusive breastfeeding duration before (regression coefficient β = -0.043, $p = 0.174$) or after (regression coefficient β = -0.010, $p = 0.678$) adjusting the model with the potential confounders obtained from the factor analysis.

Discussion

In the present study, the three inflammatory markers analyzed (TNF-α, IL-6 and CRP) correlated negatively with exclusive breastfeeding duration in adolescents but showed no

correlation with their BMI z-score. It is noteworthy that serum concentrations of inflammatory markers were much lower in individuals that had been breastfed for more than 6 months than in those breastfed for less. The greatest reduction was observed for CRP, which demonstrated a striking reduction after the fourth month of exclusive breastfeeding (see Figure 2). According to Silva, Sanches, Mello, and Damasceno (2010), the acute decline in serum levels of CRP might have a preventive effect against the early development of chronic inflammatory processes and cardiovascular disease. However, the present cross-sectional design does not allow us to conclude with certainty whether this reduction in CRP levels is due to breastfeeding.

Factor analysis showed a strong association of these three inflammatory markers with variables related to breastfeeding, including birth weight, maternal smoking, fasting glucose levels and breastfeeding. Multivariate correlations with breastfeeding duration were statistically significant for the three markers and were maintained after adjustment for all meaningful variables obtained from the factor analysis (i.e., sex, age, body fat, weight at birth, fasting glucose, fasting insulin, waist circumference, BMI z-score, maternal smoking during pregnancy and four skinfold thicknesses).

Relatively few studies have addressed the relationship between breastfeeding duration and low-grade inflammatory markers. Causal relationships are difficult to detect because prospective studies are impractical to carry out due to ethical reasons, so knowledge is usually gathered from cross-sectional studies. Vérier et al. (2011) stratified 484 healthy adolescents from diverse European origins according to breastfeeding duration and failed to find an association between breastfeeding duration and inflammatory status except for a slight difference in high-sensitivity CRP and L-selectin levels between subjects who had never been breastfed and those who received maternal milk for more than 6 months. The discrepancy between their results and ours may be a consequence of differences in sample

size, population characteristics or the number of subjects with extended lactation, which was high in our study. Martin et al. (2005) also did not find any relationship between breastfeeding and inflammation in 1062 adult men. Vérier et al.'s and Martin et al.'s results are in contrast to those of Williams, Williams, and Poulton (2006), who found a significant linear relationship between breastfeeding duration and CRP levels in 26-year-old women who had been breastfed for at least 6 months. Also, in a study of 9337 subjects, Rudnicka, Owen, and Strachan (2006) found that breastfeeding for at least 1 month was associated with lower CRP and fibrinogen in women. More recently, Labayen et al. (2012) reported that exclusive breastfeeding in infancy was associated with lower serum fibrinogen levels in children and adolescents.

In a meta-analysis designed to determine risk factors for childhood overweight during the first year of life, authors reported a moderate protective effect (15%) of breastfeeding on subsequent childhood overweight (Weng, Redsell, Swift, Yang, & Glazebrook, 2012). Obesity is considered to be a chronic low-grade inflammatory process (Trayhurn, Bing, & Wood, 2006), characterized by an increase in some inflammatory markers released by adipocytes, such as CRP (Aronson et al., 2004) and TNF-α, IL-6 (Hotamisligil, Shargill, & Spiegelman, 1993), and even the infiltration of macrophages in adipose tissue (Weng et al., 2012). In children, obesity has been associated with higher CRP levels (Choi, Joseph, & Pilote, 2013), and there is evidence indicating that IL-6 and TNF-α might be elevated as well (Street et al., 2013). In the present study, body weight did not affect the association between breastfeeding duration and inflammatory markers except for a significant difference between normal-weight and obese adolescents in IL-6 concentration. This finding might have been influenced by the low number of obese individuals in our sample.

Weight at birth has also been associated with higher levels of chronic inflammation. In a group of 166 Swedish children and 126 adolescents, researchers observed negative adjusted

associations of birth weight with fibrinogen and complement factors C3 levels but not with CRP levels (Labayen, Ortega, Sjöström, & Ruiz, 2009). We did not perform correlations of birth weight and inflammatory markers in the present study, but birth weight exhibited a strong influence in factor analysis. Therefore, we included this parameter in the regression model for adjustments. However, these data should be interpreted with caution since only 3.8% of participants did not receive breast milk as infants.

In fact, duration of exclusive breastfeeding was surprisingly high in our study, with a mean of 9.3 ± 2.7 months. This duration is higher than expected for the Spanish population, according to Colodro-Conde et al. (2011). However, researchers have reported prolonged breastfeeding (mean duration higher than 9 months) in Hispanic populations in the United States (Eskenazi et al., 2011), where up to 21.7 % of children are breastfed for 6 months or longer (Gill, 2009). Breastfeeding duration is associated with socioeconomic status (Kwok, Schooling, Lam, & Leung, 2010) and maternal educational level (Colodro-Conde et al., 2011). Colodro-Conde et al. showed that, for a Spanish population, breastfeeding duration was higher in mothers with a higher educational level, which is also true for exclusive breastfeeding (Jessri, Farmer, Maximova, Willows, & Bell, 2013) and is consistent with findings in the present study. Indeed, we found a strong positive correlation between maternal educational level and breastfeeding duration ($r = 0.836$, $p < .001$), which might explain the prolonged duration of breastfeeding in our study. Socioeconomic status and educational level of the parents are also related to inflammation in adolescents (Chiang et al., 2015). The inclusion of these variables as confounders in their analysis might be why Vérier et al. (2011) did not find an association between breastfeeding and inflammation. We did not include socioeconomic status (the data were unavailable) in our analyses in the present study, but we did include maternal educational level. When we included this latter variable in the factor analysis, we found that it was closely associated with breastfeeding (see supplementary

table).

The mechanisms by which breastfeeding influences the inflammatory status of adolescents remain unclear, but it has been hypothesized that this relationship is mediated by breastfeeding's effect on body weight in adolescence, as BMI at this age is related to enhanced systemic inflammation (Das, 2001). However, the influence of breastfeeding duration on obesity-related inflammation is still controversial (Labayen et al., 2012; Singhal et al., 2004; Vérier et al., 2011). In this regard, our results show no significant association between BMI in adolescents and breastfeeding duration in their infancy.

Another possibility for the association between breastfeeding and inflammation in adolescents might be related to the developmental origins of health and disease. Accumulating evidence suggests that nutrition during pregnancy and early postnatal life is one of the most important environmental cues that program metabolic development. This factor influences growth and development during the sensitive postnatal period, which has long-term consequences, including later health problems such as metabolic syndrome, cardiovascular disease and obesity (Tarry-Adkins & Ozanne, 2011). Events or phenomena like colonization of gut microbiota in early stages of life (Nauta, Ben Amor, Knol, Garssen, & Van der Beek, 2013), dietary fatty acid intake during lactation (Innis, 2011), or maternal obesity (Heerwagen, Miller, Barbour, & Friedman, 2010) can lead to elevated inflammatory cytokines and leptin, which in turn influence the onset of diabetes and obesity. Epigenetics might also be involved. For instance, duration of breastfeeding has been negatively associated with leptin methylation, which could contribute to the protective effect of breastfeeding against obesity (Obermann-Borst et al., 2013).

The present study has some strengths and limitations. Among the strengths, the number of participants in this study is high compared to other recent studies that have examined the effect of breastfeeding on inflammation. In addition, all participants belonged

to the same geographical region, with similar cultures, lifestyles and dietary habits, making the sample more homogeneous. We consider the study to have great relevance from an epidemiological point of view because the recruitment strategy was designed to create a representative sample of the entire population for the age groups studied in the region, which allowed us to obtain results that could be generalizable to other populations. We included confounders such as age, sex, weight at birth, maternal smoking during pregnancy, maternal educational level and maternal obesity in the regression model. However, we did not include other potential confounders such as socioeconomic status or physical activity. Nevertheless, all participants were of a similar socioeconomic status in that they attended high schools in middle-class areas . We recorded breastfeeding duration in months rather than weeks, which limits the precision of the correlations. Further, we gathered the data from parents using a questionnaire, which might lead to a recall bias. Finally, we cannot dismiss the fact that 77% of the participants had been breastfed for 9 months or more, which might reduce the chances of observing differences.

In conclusion, results of the present study indicate a potential relationship between the inflammatory markers TNF-α, IL-6 and CRP and the duration of exclusive breastfeeding in Spanish adolescents. This relationship appears not to be mediated by body weight or BMI, suggesting that the relationship between breastfeeding and inflammation is not mediated by the effect of increased body weight or obesity. Rather, the mechanism might be independent factors operating through prenatal or early-life metabolic programming and should be further investigated. Our results represent a potentially significant advance in nursing knowledge because by increasing our understanding of the associations between breastfeeding and the inflammatory processes inherent in obesity, we increase our ability to develop interventions that promote healthy adolescence.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Figure Captions

Figure 1. Principal component analysis loading plot. Filled circles indicate variables related to body-weight (body mass index [BMI]) related; unfilled circles indicate variables related to breastfeeding. HOMA-IR = homeostatic model assessment for insulin resistance; WHR = waist-to-hip ratio.

Figure 2. Serum (A) tumor necrosis factor (TNF)-α, (B) interleukin (IL)-6 and (C) C-reactive protein (CRP) concentrations in adolescents according to exclusive breastfeeding duration. $p < .001$ for nonadjusted regression analysis.

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Table 1. Demographic and anthropometric characteristics of the participants (N = 1,001).

Characteristic	Mean ± SD or %
Age (years)	13.2 ± 1.2
Sex (% female)	52.1
Waist-to-hip ratio	0.86 ± 0.06
Waist circumference (cm)	72.28 ± 10.79
Body fat (%)	28.53 ± 8.08
Skinfold thickness (mm)	
Bicipital	9.18 ± 84.72
Tricipital	17.16 ± 7.10
Suprailiac	17.52 ± 9.47
Suprascapular	12.82 ± 7.20
BMI (kg/m^2)	21.23 ± 3.77
BMI classification	
Normal weight	85.3
Overweight	10.1
Obese	4.6
Breastfeeding duration	
None	3.8
< 3 months	3.6
≥ 3 to < 6 months	3.7
≥ 6 to < 9 months	12.0
Breastfeeding ≥ 9 months	76.9
Mother smoked during pregnancy	21.4
Weight at birth (kg)	3.20 ± 0.50
Blood pressure (mmHg)	
Diastolic	64.1 ± 9.0
Systolic	118.0 ± 15.5

Note. BMI = body mass index.

Table 2. Serum concentrations of inflammatory markers in adolescents according to their body mass index (BMI) category.

Marker	Normal Weight (n = 851)	Overweight (n = 103)	Obese (n = 47)	p-value
TNF-α (pg/mL)	11.78 ± 2.48	11.81 ± 2.30	12.70 ± 3.45	.051
IL-6 (pg/mL)	12.75 ± 2.38[a]	12.75 ± 2.18	13.67 ± 3.28[a]	.039
CRP (mg/dL)	1.12 ± 1.10	1.12 ± 1.04	1.52 ± 1.48	.058

Note. Values are expressed as mean ± SD. CRP = C-reactive protein; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-alpha.

[a]Values that differ significantly from each other at $p < .05$.

Table 3. Serum concentrations of inflammatory markers in adolescents according to breastfeeding duration.

	Breastfeeding Duration		
	0−6 months	> 6 months	
Marker	(*n* = 114)	(*n* = 887)	*p*-value
TNF-α (pg/mL)	17.66 ± 3.02	11.08 ± 1.02	< .001
IL-6 (pg/mL)	18.37 ± 3.05	12.08 ± 0.95	< .001
CRP (mg/dL)	3.79 ± 1.65	0.80 ± 0.25	< .001

Note. Values are expressed as mean ± SD. CRP = C-reactive protein; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-alpha.

Table 4. Correlation parameters of inflammatory markers according to body mass index (BMI) and breastfeeding duration in adolescents using multivariate regression analysis (*N* = 1001).

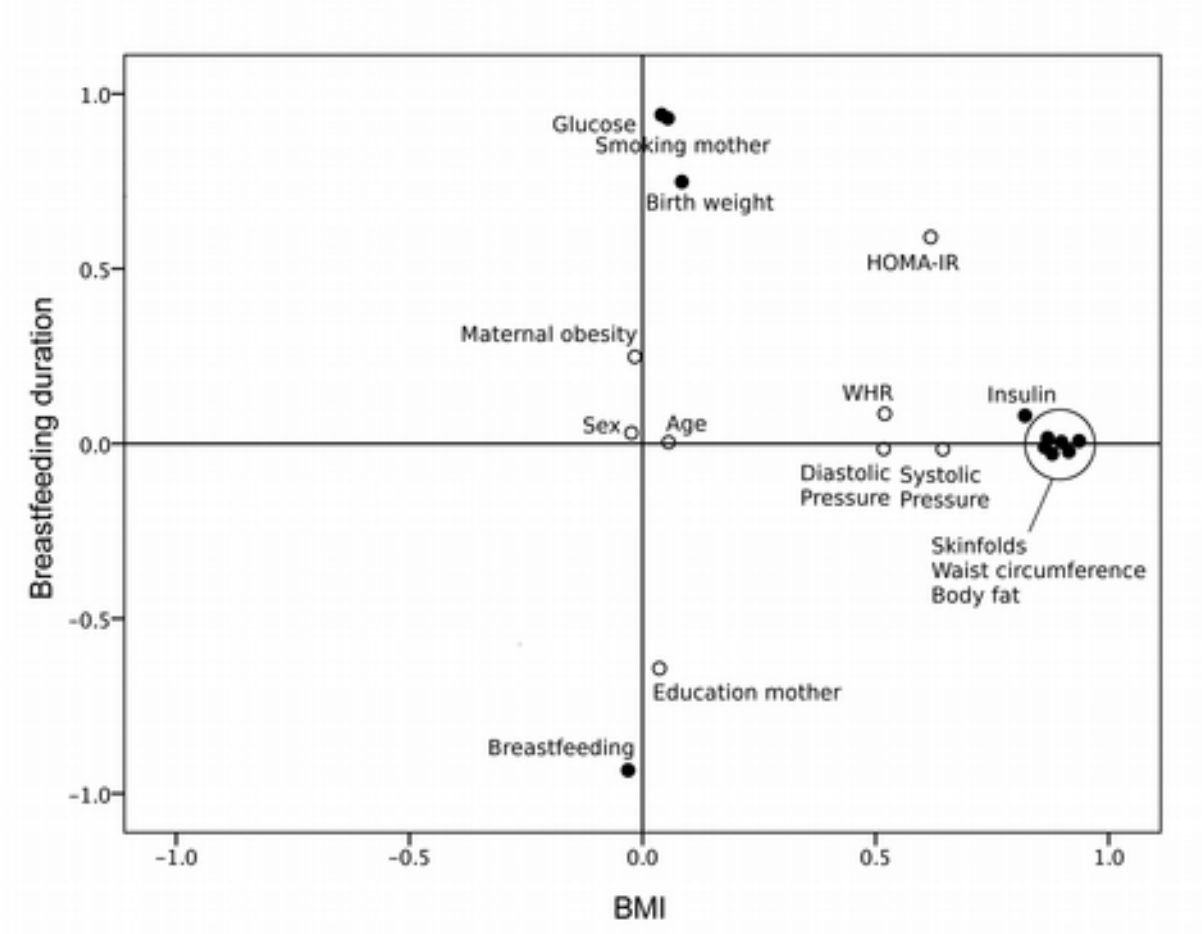
	BMI				Breastfeeding Duration		
	Nonadjusted		Adjusted		Nonadjusted		Adju
Marker	β (CI-95%)	*p*-value	β (CI-95%)	*p*-value	β (CI-95%)	*p*-value	β (CI-95%)
TNF-α (pg/mL)	0.042	.156	-0.047	.161	-0.862	< .001	-0.214
IL-6 (pg/mL)	0.050	.112	0.002	.958	-0.815	< .001	-0.321
CRP (mg/dL)	0.047	.138	0.003	.914	-0.051	< .001	-0.506

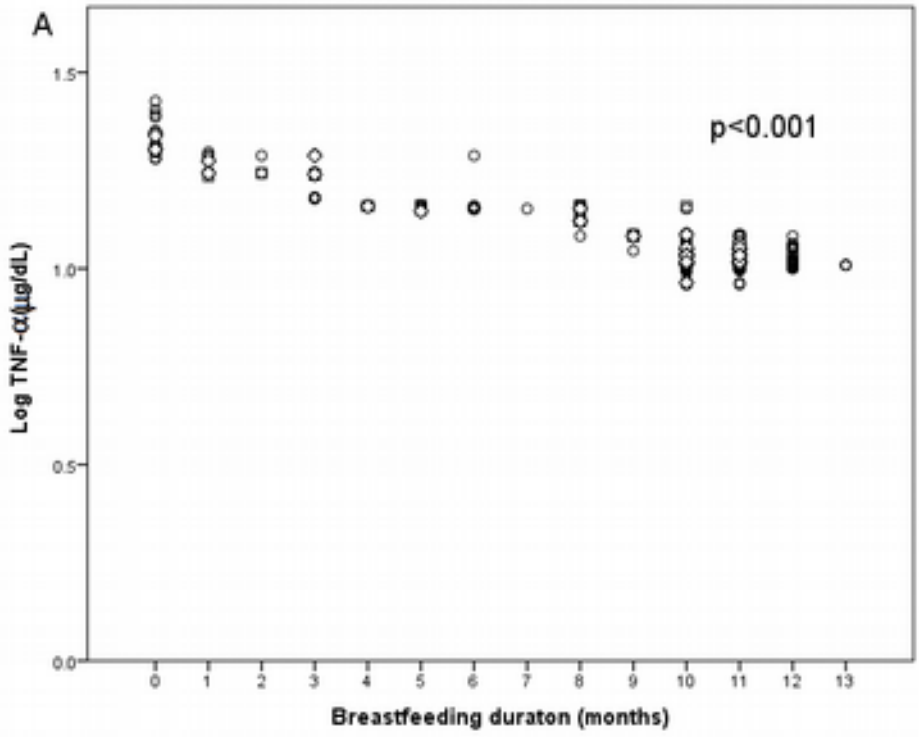
Note. The regression coefficient (β) is the effect size per unit increase in breastfeeding duration or BMI analyzed within the same model. Values are nonadjusted or adjusted for sex, age, body fat, weight at birth, fasting glucose, fasting insulin, waist circumference, BMI z-score, maternal smoking during pregnancy, and bicipital, tricipital, supraescapular and suprailiac skinfold

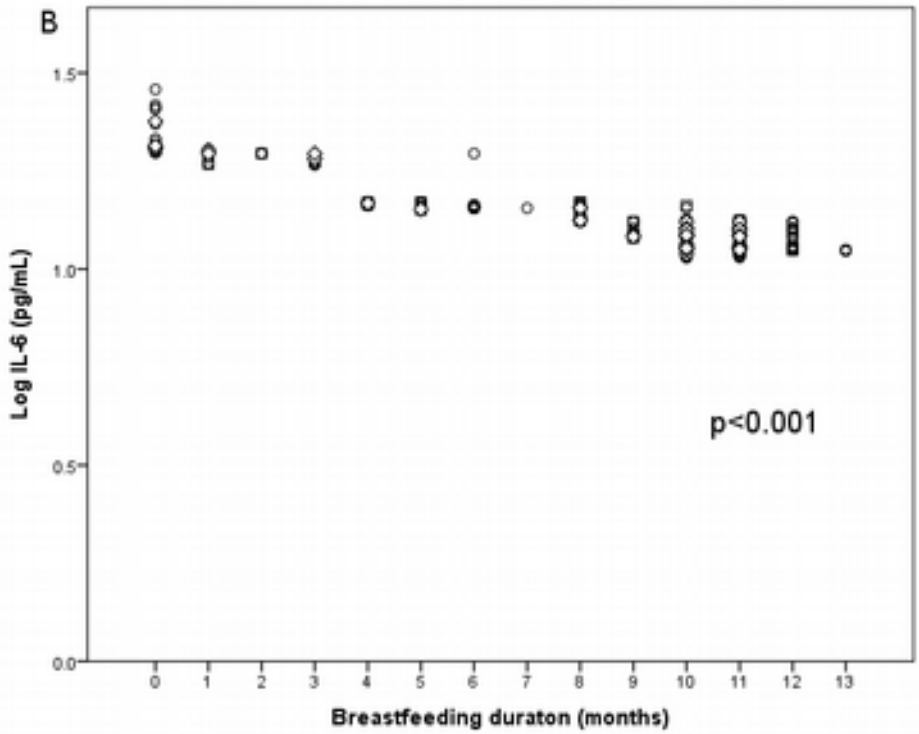
Supplementary Table. Factor loading values for variables after principal component analysis in adolescents.

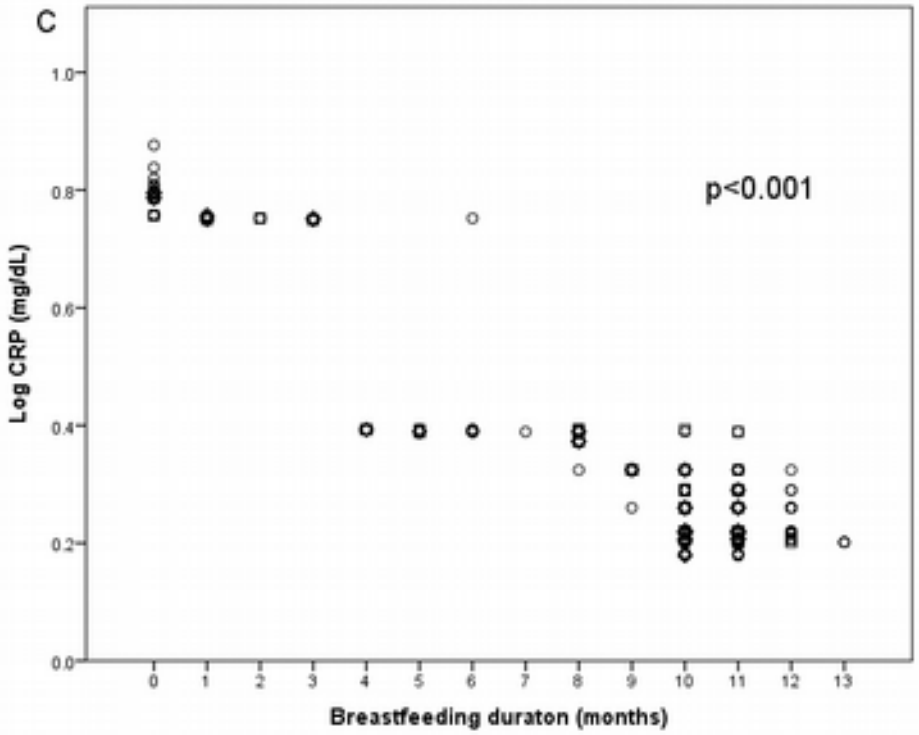
	Factor	
Variable	BMI	Breastfeeding
Sex	-0.042	0.023
Age	0.062	0.005
Body fat	0.920	0.011
Glucose	0.001	0.954
Insulin	0.817	0.099
Systolic blood pressure	0.644	0.004
Diastolic blood pressure	0.521	0.002
HOMA-IR	0.600	0.609
Birth weight	0.062	0.744
Maternal obesity	-0.020	0.252
Maternal smoking	0.006	0.947
Bicipital skinfold	0.880	0.024
Tricipital skinfold	0.903	0.034
Suprascapular skinfold	0.883	0.004
Suprailiac skinfold	0.866	0.018
Waist circumference	0.858	0.041
Maternal educational level	0.027	-0.649
BMI z-score	0.936	0.035
Breastfeeding duration	-0.012	-0.949

Note. Factor loading values of > 0.7 (or <-0.7) indicate that the variable was a major constituent of the factor. Each factor is named according to the variables that are its major constituents. BMI = body mass index; HOMA-IR = homeostatic model assessment for insulin resistance.


Glucose
Smoking mother
Birth weight
HOMA-IR
Maternal obesity
WHR
Insulin
Sex
Age
Diastolic Pressure
Systolic Pressure
Skinfolds
Waist circumference
Body fat
Education mother
Breastfeeding
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0
Breastfeeding duration
BMI


A
Log TNF-α(μg/dL)
p<0.001
Breastfeeding duraton (months)


B
Log IL-6 (pg/mL)
p<0.001
Breastfeeding duraton (months)


C
Log CRP (mg/dL)
p<0.001
Breastfeeding duraton (months)