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Breastfeeding in Infancy Is Not Associated with Inflammatory Status in Healthy Adolescents[1–3]

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Abstract

It has been suggested that breast-feeding (BF) may be associated with a decreased risk of cardiovascular disease in adulthood. A low-grade inflammation is associated with an increased risk of cardiovascular disease, even in apparently healthy children. The objective of this study was to assess the potential modulating effect of BF on the inflammatory status of healthy adolescents. Information on BF (duration) was obtained from parental records in 484 of 1040 healthy European urban adolescents (56.4% females) that had a blood sample obtained as part of the Healthy Lifestyle in Europe by Nutrition and Adolescence study. Blood serum inflammatory markers were measured, including high sensitivity C-reactive protein, complement factors 3 and 4, ceruloplasmin, adhesion molecules (L-selectin and soluble endothelial selectin, soluble vascular cell adhesion molecule 1, and intercellular adhesion molecule 1), cytokines, TGFβ1, and white blood cells. After univariate analysis, a propensity score, including the potential confounding factors, was computed and used to assess the association between BF and selected inflammatory markers. BF was not significantly associated with any of the selected inflammatory markers after adjustment for gender and propensity score. In our study, BF was not associated with low-grade inflammatory status in healthy adolescents, suggesting that the potential cardiovascular benefits of BF are related to other mechanisms than modulation of inflammation or might become relevant at a later age. Groups at high risk for cardiovascular disease should be a target for further research concerning the effects of BF. J. Nutr. 141: 411–417, 2011.

Introduction

Breast-feeding (BF)[19] is the natural and advisable way of supporting healthy growth and development of young children, as recently reemphasized by international public health authorities and scientific societies (1,2). However, methodological difficulties arise when assessing the health benefits of BF for both the infant and mother. The maternal decision to breast-feed is influenced strongly by multiple factors that may confound the analysis, such as educational level or socioeconomic status (SES), lifestyle, and smoking habits. Even in studies that controlled for known confounding variables, residual confounding factors remained. To identify a causal relationship, randomized, double-blind prospective studies are needed but are impossible to perform to study BF for obvious ethical considerations. Among the benefits of BF as an infant that appear later in life, the potential impact on obesity, blood pressure, and lipid metabolism has sparked many investigations but many ques-

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[19] Abbreviations used: BF, breast-feeding or breast-fed; C3 and C4, complement factors 3 and 4; FAS, family affluence scale; HDL-C, HDL cholesterol; HELENA-CSS, HELENA cross sectional study; hs-CRP, high-sensitivity C-reactive protein; LDL-C, LDL cholesterol; SES, socioeconomic status; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cellular adhesion molecule 1; TC, total cholesterol.

[3] Supplemental Figures 1 and 2 and Supplemental Appendix are available with the online posting of this paper at jn.nutrition.org.
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tions remain. A meta-analysis showed that BF infants have a lower risk of becoming overweight or obese in childhood (OR = 0.78; 95% CI = 0.72–0.84) (2). However, this benefit has not been shown during adulthood and the dose-response effect of BF is not consistent (3). Regardless of the age at assessment, systolic blood pressure is slightly but significantly lower in people who were BF as infants than in those who were formula fed (pooled difference: –1.4 mm Hg; 95% CI = –2.2 to –0.6) (2,4,5). Although there is evidence supporting the beneficial effects of BF on blood pressure and blood cholesterol level, there is no convincing evidence that BF in infancy has an effect on cardiovascular morbidity and mortality later in life (6,7).

A state of chronic low-grade inflammation was reported recently in apparently healthy people, including children, when assessed using new sensitive techniques for measuring biomarkers, such as high-sensitivity C-reactive protein (hs-CRP) and new inflammatory markers (8). Interestingly, the moderately elevated concentrations of inflammatory markers accelerate vascular damages and predict vascular risks from an early age (9–11). A review concluded that systemic low-grade inflammation correlates with obesity in childhood and plays a pathogenic role in insulin resistance, atherosclerosis, and possibly nonalcoholic fatty liver disease (12). The inflammatory cascade could also be involved in the later development of high blood pressure (13,14).

Modulating low-grade inflammation might be one mechanism responsible for the beneficial effects of BF in infancy on obesity and hypertension later in life. Das et al. (15) suggested that breast milk, a rich source of long-chain PUFA, might decrease inflammation through mechanisms including statins, PPARγ binding agents, or nonsteroidal antiinflammatory agents. Few studies have investigated the influence of BF on markers of low-grade inflammation, and those that have done so have focused on specific populations. Williams et al. (16) found a significant inverse linear correlation between the duration of BF and CRP level in 26-y-old New Zealand women who had been BF for at least 6 mo. In a randomized study comparing feeding with human milk and formula in preterm infants, Singhal et al. (17) found a significantly lower CRP concentration in 13- to 16-y-old British adolescents who were BF while preterm than in those fed a preterm formula.

The objective of our study was to evaluate whether BF in infancy influences markers of low-grade inflammation in healthy adolescents.

Materials and Methods

Study design. This report is based on data derived from the Healthy Lifestyle in Europe by Nutrition in Adolescence cross-sectional study (HELENA-CSS), which aims to obtain a broad range of standardized, reliable, and comparable nutrition- and health-related data from a random sample of European adolescents. Data collection took place during 2006 and 2007 in 10 European cities: Athens (Greece), Dortmund (Germany), Ghent (Belgium), Heraklion (Greece), Lille (France), Pecs (Hungary), Rome (Italy), Västeras (Sweden), Vienna (Austria), and Zaragoza (Spain). The adolescents studied were residents of the 10 selected cities. A detailed description of the HELENA-CSS sampling and recruitment approaches, standardization and harmonization processes, data collection, analysis strategies, and quality control activities was published elsewhere (18,19).

All the adolescents meeting the general HELENA inclusion criteria (not participating simultaneously in another clinical trial) and having valid data for age, sex, and BMI were considered the final HELENA-CSS sample (3528 adolescents aged 12.5–17.5 y). One-third of the participants were randomly selected to provide data from the assays of the blood sample for clinical biochemistry assays and genetic analyses (n = 1089; **Supplemental Fig. 1**). A total of 484 adolescents with valid data from the assays of the blood sample (n=49 excluded) and duration of BF (n=108 excluded) were included in the present study. Adolescents who presented with an acute infection lasting <1 wk before the inclusion (n= 307) or who had fever (>38°C) during the 24 h before the blood sample (n=141) were excluded. We verified that the BMI, age, sex, and SES were similar between the 484 children included and the 108 children who had a blood sample but no valid data on BF (**Table 1**). The final sample of 484 children was equally distributed among the cities: Athens (n = 37), Dortmund (n = 59), Ghent (n = 32), Heraklion (n = 45), Lille (n = 53), Pecs (n = 74), Rome (n = 51), Västeras (n = 49), Vienna (n = 45), and Zaragoza (n = 39). After receiving comprehensive information on the study's aims and methods, all adolescents and their parents or guardians signed an informed consent form. The protocol was approved by the investigational review boards at the participating university medical centers (20).

Neonatal assessment. Data on weight and height at birth and the duration of gestation and of BF (in months) were collected via a parental questionnaire, as reported elsewhere (21). The duration of gestation was stratified into 3 categories: <37 wk of amenorrhea (preterm infants), between 37 and 42 wk of amenorrhea, and >42 wk of amenorrhea (post-term infants). The total duration of BF was classified into 4 categories: never BF, <3 mo, ≥3 to <6 mo, and ≥6 mo (22). The duration of exclusive BF, defined by the WHO as no liquid or solid nutrition other than breast milk (2), was coded in a similar manner.

Inflammatory markers. Several inflammatory markers involved in low-grade inflammation were selected (23): hs-CRP, which is associated with low-grade inflammation and cardiovascular risk (24) in obese and nonobese children (25); complement factors 3 and 4 (C3 and C4) (25); ceruloplasmin (low-grade inflammation marker in females only) (26); adhesion molecules L-selectin and sE-selectin; and endothelial function markers: soluble vascular cell adhesion molecule-1 (sVCAM-1) and intercellular adhesion molecule-1 (sICAM-1), cytokine TGFβ1, and white blood cells (27).

hs-CRP and ceruloplasmin were measured in serum by immunoturbidimetry (AU2700 biochemistry analyzer; Olympus); the stability of samples during transport and storage has been reported previously (28). C3 and C4 serum complement was analyzed by nephelometry (Behring Diagnostics). The total plasma C3 values in our study represented C3, C3b, and C3c production. The CV (inter-assay precision) were ≤2% for all proteins (1.90% for hs-CRP, 2.00% for ceruloplasmin, 1.39% for C3, and 1.19% for C4). Detection limits (sensitivity) for the analyses were 0.007 mg/L for hs-CRP, 0.04 g/L for ceruloplasmin, 0.01 g/L for C3, and 0.002 g/L for C4. Single-point measurement was performed for all the analyses of acute phase proteins, except for hs-CRP, which was analyzed in duplicate. Serum adhesion molecule sL-selectin and sE-selectin (g/L) were analyzed using commercial ELISA kits (Diaclone) on the Universal Microplate spectrophotometer (Power WaveTM XS, Biotek Instruments). The sensitivity was <1.0 g/L. Measurement of serum soluble sE-selectin, sVCAM-1, and sICAM-1 (all g/L) was performed with Luminex-100 IS (Integrated System: Luminex) technology by using the

TABLE 1 Characteristics of the 108 children who had a blood sample but no valid BF data and of the 484 children included in the study[1]

Main confounders	Adolescents included, n = 484	No valid data on BF, n = 108	P-value[2]
Girls, *n*	273.0	40.0	0.004*
Age, *y*	14.7	14.9	0.14
BMI, *kg/m²*	21.6	21.8	0.60
FAS 4 items, *number of facilities*	4.4	4.4	0.67

[1] Other than gender, values are means from the bivariate analysis. *Infirmed by multivariate analysis.
[2] Pearson correlation coefficient was used for continuous variables and ANOVA for categorical variables.

multiplex assay kit Linco Human Cardiovascular Disease (CVD) Panel 1 Lincoplex, 96 Well Plate Assay (HCVD1-67AK), manufactured by Linco Research. Multianalyte profiling calibration microspheres for classification and reporter readings, as well as sheath fluid, were also purchased from Luminex Corporation. Acquired fluorescence data were analyzed by the Luminex 2.3 version software. All analyses were performed according to the manufacturer's protocols. The sensitivities were 79.0 ng/L for sE-selectin, 16.0 ng/L for sVCAM-1, and 9.0 ng/L for sICAM-1. The intra- and inter-assay precision CV were: 11.2 and 13.4%, respectively, for sE-selectin; 4.5 and 8.5%, respectively, for VCAM-1; and 7.9 and 9.7%, respectively, for ICAM-1. Single-point measurement was performed for the analyses of all adhesion molecules. Serum TGFβ1 levels were measured using commercial ELISA kits (Diaclone) and analyzed by the Universal Microplate spectrophotometer (Power WaveTM XS, Biotek Instruments). The intra-and inter-assay precision CV were 6.7 and 8.5%, respectively. A pilot study was performed to confirm the reliability of our measurements.

Potential confounding factors. We focused on potential confounding factors that might influence the relationship between inflammatory status and BF. Many confounding factors have been reported, including acute infection, gender (29), birth weight (30), age and pubertal status (31), BMI and waist circumference (32), physical activity (33,34) and fitness (35), systolic and diastolic blood pressure (13,36), SES (37), active smoking exposure (38), oral contraceptive use in girls (39), and blood lipid concentration (40–42).

Systemic inflammation differs between the genders (27). The harmonized, standardized anthropometric measurements used to assess body composition in the HELENA study were monitored strictly and have been described previously (43). Body weight was measured with an electronic scale (Seca 861) to the nearest 0.1 kg and height was measured with a stadiometer (Seca 225) to the nearest 0.1 cm. BMI was calculated as body weight in kg divided by the square of height and is expressed as kg/m^2. Waist circumference was measured with a nonelastic tape. Identification of sexual maturation (Tanner and Whitehouse stages I–V) was assessed by a physician (44). The scale runs from stage I (prepubertal status) to stage V (complete maturation). Oral contraceptive use in girls was recorded. Systolic and diastolic blood pressure were measured twice after the participant sat quietly for 5 min (Omron M6, UK; 4 cuff sizes according to age and weight); the lowest value was recorded in the database.

Physical activity and sedentary behavior were evaluated over a 1-wk period using an accelerometer (ActiGraph MTI, model GT1M, Manufacturing Technology), an accurate device for assessing daily physical activity. Physical activity was recorded in counts per minute and coded as low, moderate, or vigorous physical activity (45). Physical fitness was assessed by the 20-m shuttle-run test, a cardiorespiratory fitness test in which the participants are encouraged to run for as long as possible between 2 lines 20 m apart (46). The scientific rationale and the reliability of these tests in young people have been published previously by our group (47).

SES was assessed by both the maternal educational level (coded into 4 categories: elementary, lower secondary, higher secondary, or higher education) and the family affluence scale (FAS). This latter scale is the sum of conditions of living, such as "facilities" and "consumption" indicators, and is more suitable for adolescence than is the maternal educational level, which is a better indicator of SES in infancy (48). Two versions of the FAS were used. FAS 3 includes the number of bedrooms in the house and cars and computers owned by the family, and FAS 4 includes the same variables plus the number of Internet connections at home. Adolescent smoking habit was assessed by the number of cigarettes smoked per week.

Blood samples were drawn after a 10-h overnight fast according to a standardized collection protocol and were sent to a central laboratory (Analytical Laboratory, Institut für Ernährungs-und Lebensmittelwissenschaften) for subsequent biochemical measurements (42). Blood samples were shipped and analyzed by the Central Laboratory within 24 h. The following blood lipid levels were measured in fresh serum sample: total cholesterol (TC), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), TC:HDL-C ratio, HDL-C:LDL-C ratio, ApoA-1 and ApoB, ApoB:ApoA-1 ratio, and TG (cg/Lf). Fresh serum samples were used for the analysis. TG, HDL-C, LDL-C, and TC concentrations were measured using the Dimension RxL clinical chemistry system (Dade Behring) and ApoA-1 and ApoB concentrations were measured using an immunochemical method on a BN II analyzer (Dade Behring).

Statistical methods. Continuous variables are expressed as mean and SD and qualitative variables as frequency and percentage. Because the distribution of hs-CRP concentration was strongly skewed, all analyses involving hs-CRP were performed after rank transformation, and the results for hs-CRP are expressed as median and interquartile range.

The relationships among the 4 categories of BF duration and inflammatory markers were tested using ANOVA. The duration of BF at some time differed noticeably between the 10 cities involved in the study and the analyses were adjusted for geographical location. Because of the presence of numerous potential confounding factors, we performed further analysis. Some of the biomarkers differed among the BF duration groups. Because there can be a recall bias about the exact duration of BF, we decided to use only the extreme 2 categories of BF duration in the adjusted analysis: never BF and BF $\geq$ 6 mo.

A bivariate analysis was performed between each potential confounding factor (sociodemographic factors, clinical factors, and physical activity and sedentary behavior data) and selected inflammatory markers in the 2 BF groups. Selected inflammatory markers were analyzed because they had a P-value $\leq$ 0.10 in the previous analysis. Comparisons between only the groups never BF and BF $\geq$ 6 mo were also performed for all inflammatory markers, and markers having a P-value $\leq$ 0.10 were selected for further analysis.

The adjustment for confounders was performed by using the propensity score method. This method is a 2-step procedure. In a first step, a logistic regression is performed with BF considered as dependent variable and the confounders as independent variables. The propensity score is the probability of being BF estimated using the logistic model. The propensity score reflects the differences between the 2 groups (BF $\geq$ 6 mo vs. never BF) on the confounders. In a second step, the effect of BF on a marker is adjusted for the propensity score using a covariance analysis. The propensity score method provides better results as the multivariable linear regression for adjustment on confounders. It is indeed not subject to the problem of over-parameterization, which occurs when numerous confounders exist and which consequently could conduct a biased estimation of the effect of interest, BF (50).

To account for oral contraceptive use in girls, the propensity scores were computed separately according to sex. We did not include all the lipid variables, because they strongly correlated with each other.

The data were analyzed using SAS software version 9.1 (SAS Institute). In final multivariate analyses using propensity score, P-values < 0.05 were considered significant. In intermediate bivariate analyses to select inflammation markers and confounders, P-values $\leq$ 0.10 were considered significant.

Results

Among the 484 adolescents included in this study, 81.4% were BF at some time (independent of duration and exclusivity); 88% of these had been BF exclusively and 24.6% were BF $\geq$ 6 mo (**Table 2**). Serum hs-CRP concentrations were within the normal range (<3 mg/L) (8) in all adolescents (**Table 3**).

We first analyzed the relationships between inflammatory markers and BF duration (Table 3). When BF duration was coded into 4 categories, only the C3 concentration was associated with BF (P = 0.03; **Supplemental Fig. 2***A*). The serum hs-CRP concentration tended to be related to BF duration (P = 0.1; Supplemental Fig. 2*B*). The results were similar for those who had been BF exclusively (data not shown).

When BF duration was coded into 2 categories (never BF vs. BF $\geq$ 6 mo), the hs-CRP concentration remained significantly associated with BF duration, but the C3 concentration did not.

The serum L-selectin concentration tended to be associated with BF duration (P = 0.10; Supplemental Fig. 2*C*). The other inflammatory markers did not differ significantly between the never BF and BF ≥ 6 mo groups ($P > 0.3$).

Confounders associated with the hs-CRP level were age, oral contraceptive use in girls, and serum HDL-C and TG concentrations. The L-selectin concentration was significantly associated with FAS 3, duration of gestation, HDL-C concentration, and running speed in the physical fitness test.

In the multivariate analysis, after adjusting for the propensity score, BF duration was no longer associated with the hs-CRP concentration (P = 0.32) or the L-selectin concentration (P = 0.85). We verified that results for the multi-variable association by gender also were not significant (L-selectin: P = 0.71 for boys, P = 0.58 for girls; hs-CRP: P = 0.18 for boys and P = 0.78 for girls).

Discussion

In the present study, adolescents who had been BF as infants for ≥6 mo did not have lower circulating inflammatory marker concentrations than those who had never been BF. Among the inflammatory markers tested in the univariate analysis, only hs-CRP and L-selectin levels were associated with BF classified into the 2 extreme duration categories.

These results contrast with 2 previous reports suggesting an influence of BF on inflammatory status. Singhal et al. (17) performed a randomized study to compare the use of human milk and preterm formula in preterm babies in the early 1980s. In the 13- to 16-y-old adolescents born prematurely, CRP concentrations inversely correlated with the intake of human milk in infancy ($r = -0.16$; P = 0.03). However, the population included only preterm babies (mean gestation age: 31 wk), which represent <10% of the European newborn population (51). Williams et al. (16) found a significant linear relation between BF duration and CRP level in 26-y-old women in New Zealand. In our study, inflammatory status did not differ between adolescent boys and girls. These results contrast with those published by Pirkola et al. (27), who reported differences in hs-CRP in males compared with females. In this study, mean age in girls was older than in our study (16 vs. 14.2 y). Indeed, in

TABLE 2 Association between confounders and selected inflammatory markers in healthy adolescents who were not BF or were BF ≥ 6 mo in infancy[1]

Potential confounding factors	Never BF, n = 90	BF ≥ 6 mo, n = 119	P-value[2]	P-value of selected confounding factor	
				hs-CRP	L-selectin
Age, *y*	14.7 ± 1.3	14.6 ± 1.3	0.83	0.02*	0.73
Girls, %	43	57	0.10	0.26	0.42
BMI, *kg/m*^{2}	21.4 ± 3.9	21.1 ± 3.4	0.31	0.37	0.97
Waist circumference, *cm*	73.1 ± 9.4	72.1 ± 9.5	0.32	0.45	0.97
Pubertal status, %					
Tanner stage 2	8	6			
Tanner stage 3 or 4	70	70	0.86	0.80	0.45
Tanner stage 5	22	24			
Oral contraception (girls only), %	13	8	0.35	0.05*	0.68
Mother's education, %					
Elementary school	10	5			
Lower secondary	33	23	0.15	0.87	0.25
Higher secondary	31	37			
Higher education	25	35			
FAS 3 items, *number of facilities*	3.9 ± 1.5	3.8 ± 1.6	0.40	0.86	0.08*
FAS 4 items, *numbers of facilities*	4.7 ± 1.8	4.5 ± 1.9	0.62	0.93	0.10
Smoking, *cigarettes/wk*	1.4 ± 1.1	1.2 ± 0.7	0.26	0.23	0.23
Duration of gestation, %					
37 wk of amenorrhea	12	2	0.01		
37-42 wk of amenorrhea	60	64		0.35	0.05*
>42 wk of amenorrhea	29	35			
Birth weight, kg	3.2 ± 0.7	3.4 ± 0.5	0.03	0.91	0.60
Systolic blood pressure, *mmHg*	117 ± 16	116 ± 12	0.61	0.77	0.24
Diastolic blood pressure, *mmHg*	65 ± 91	64 ± 8	0.53	0.25	0.69
Serum HDL-C,[3] *cg/L*	55.8 ± 9.4	55.2 ± 11.1	0.63	0.03*	0.09*
Serum TG,[4] *cg/L*	73.7 ± 34.8	74.1 ± 39.6	0.93	0.07*	0.52
Serum ApoB/ApoA-1	0.4 ± 0.1	0.4 ± 0.1	0.22	0.31	0.62
20-m shuttle run speed, *km/h*	10.5 ± 1.3	10.0 ± 1.2	0.02	0.72	0.08*
Accelerometer, *counts/min*	455 ± 173	421 ± 124	0.22	0.78	0.27
Time spent low physical activity, *min/wk*	529 ± 106	544 ± 93	0.40	0.95	0.59
Time spent vigorous physical activity, *min/wk*	19 ± 13	17 ± 11	0.48	0.78	0.50

[1] Values are mean ± SD or percentage from the bivariate analysis.
[2] Pearson correlation coefficient was used for continuous variables and ANOVA for categorical variables. P-values used to compute the propensity scores.
[3] 1 mg/dL = 0.0259 mmol/L.
[4] 1 mg/dL = 0.0113 mmol/L.

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TABLE 3 Serum concentrations of inflammatory markers in healthy adolescents who were not BF, BF < 3 mo, BF ≥ 3 but < 6 mo, or were BF ≥ 6 mo in infancy[1]

Inflammatory marker	Never BF 18.6%, *n* = 90	BF < 3 mo 32.2%, *n* = 156	BF ≥ 3 mo < 6 mo 24.6%, *n* = 119	BS > 6 mo 24.6%, *n* = 119	*P* (4 categories)	*P* Never BF vs. BF ≥ 6 mo
hs-CRP, *mg/L*	0.6 (0.3; 1.4)	0.6 (0.2; 1.4)	0.7 (0.3; 1.7)	0.4 (0.2; 1.1)	0.10*	0.07*
C3, *g/L*	1.12 ± 0.20	1.17 ± 0.20	1.14 ± 0.20	1.10 ± 0.20#	0.03*	0.34
C4, *g/L*	0.20 ± 0.10	0.22 ± 0.10	0.21 ± 0.10	0.21 ± 0.10	0.27	0.90
L-selectin, *μg/L*	3621 ± 1429	3745 ± 1503	3917 ± 1379	3992 ± 1763	0.29	0.10*
TGFβ1, *μg/L*	109 ± 55	99 ± 57	95 ± 58	108 ± 65	0.23	0.89
sE-selectin, *μg/L*	39 ± 22	39 ± 20	38 ± 21	36 ± 16	0.74	0.43
sVCAM-1, *μg/L*	1323 ± 417	1286 ± 387	1347 ± 457	1336 ± 440	0.67	0.84
sICAM-1, *μg/L*	174 ± 96	162 ± 85	152 ± 68	172 ± 114	0.27	0.89
Ceruloplasmin, *mg/L*	26 ± 3	27 ± 3	152 ± 3	27 ± 3	0.65	0.30
White blood cells, 1×10^9 *cells/L*	6.5 ± 1.6	6.3 ± 1.5	6.4 ± 1.5	6.3 ± 1.6	0.26	0.44

[1] Other than hs-CRP, values are mean ± SD from the ANOVA; hs-CRP is expressed as median [inter-quartile range]. #Differs from the other 3 values in the row, $P < 0.05$. **P*-values used to compute the propensity scores.

our study, only ~25% of the girls had finished puberty at the time of the study, meaning that for 75% of the girls, menstrual cycle could not influence inflammatory markers. These differences may explain contrasts in results.

Our study has several strengths and limitations. A strength is the European extension of the multicenter study design, which offered the opportunity to analyze a sample of 484 European adolescents. The design of the HELENA study allowed us to verify several times that the adolescents did not have any underlying infection, which is a major confounding factor when studying inflammatory markers (307 adolescents excluded). Potential new adjustments and a large panel of inflammatory markers could be included in the study based on recent data. In addition, the propensity score allowed us to analyze several potential confounding factors without losing statistical power. One limitation of our study is the cross-sectional design. Despite the precautions taken, we could not assess all possible confounding factors, such as parental weight, food preference (52), and smoking habits (53), and their potential effects cannot be excluded completely. We verified that the final sample of 484 adolescents was similar to the 108 adolescents with no valid data on BF (Table 1), but we cannot formally exclude residual selection bias (gender significance was infirmed later in multivariate analyses). However, the 2 groups did not significantly differ. Parental recall of BF at least 12 y later introduces an obvious memory bias (54). However, in several centers, we had access to the child health booklet and checked the validity of the data obtained from the parental questionnaire. Another limitation was that the duration of BF was recorded in months and not weeks and as categories and not numerical continuous variables. The recall bias would indeed influence categorization of participants, especially in intermediate BF duration categories. That is why we decided to compare the 2 extreme groups, never BF and BF ≥ 6 mo. These categories are easy for parents to correctly identify and comparing the extremes would enable us to obtain clearer results. The phenotype of being small for gestational age, which can influence later inflammatory marker levels, was not assessed (55) but was probably very low, as was the percentage of preterm children in our sample (4.6%). Although the levels of several inflammatory markers known to be linked with cardiovascular risk [e.g. IL-6, IL-1, TNFα, fibrinogen, sialic acid, plasminogen activator inhibitor-1 (23), and factor VII (55)] were not available in the present study, the HELENA data included a large range of sensitive inflammatory markers such as hs-CRP, which were entered in the univariate analysis. Previous studies of BF analyzed only the CRP concentration (16,17). Finally, because we found no relationship between the inflammatory markers and BF duration, one might question the power of this study. Assuming that the hs-CRP concentration has an SD = 1 (according to observed values), the sample size of this study would have allowed us to detect a difference of 0.4 mg/L between the 2 groups.

There are several possible explanations for the absence of an association between BF in infancy and inflammatory markers in adolescence. BF might influence low-grade inflammation only in people who are genetically predisposed to cardiovascular disease. For instance, GG carriers of the 866G>A polymorphism of the UCP2 gene have higher fibrinogen, C3, and C4 levels than do people carrying the A allele (UCP2 -866G>A polymorphism) (56). Also, BF might affect inflammatory markers only in subgroups of the population who have more pronounced low-grade inflammatory status (51). Compared with the general population, people with a specific phenotype, such as being obese or overweight (57), of low birth weight (58), or exposed to environmental factors such as passive smoking (59), may exhibit low-grade inflammation. Preterm infants could be differently influenced by BF compared with full-term infants, because it has been suggested that preterm infants have higher low-grade systemic inflammation. Labayen et al. (60) showed that low birth weight was associated with chronic low-grade inflammation in children and adolescents. It has also been suggested that both small size at birth and excessive weight gain during adolescence and young adulthood may predispose to low-grade inflammation (30). Biological mechanisms are unclear, possibly with metabolism regulations. A stress response, programming during pregnancy or early life, could also lead to an activated inflammatory response still later in life. Fibrinogen and CRP are both acute phase reactants secreted by the liver and concentrations are higher when animals are growth restricted in utero (61). It has also been shown that very preterm birth is associated with higher blood pressure and a less favorable fat distribution and perhaps with a higher low-grade inflammation (62). In this high-inflammation risk group of patients, BF should limit inflammation but should not affect full-term infants. Another hypothesis is that adolescence is too early in life to detect a significant benefit of BF in infancy on cardiovascular risk. Williams et al. (16) found a significant inverse linear correlation between the duration of BF in infancy and the CRP level in adulthood, when

inflammation is more pronounced because of toxic, hormonal, or environmental pejorative factors. Finally, the cardiovascular benefits of BF in infancy might also be related to mechanisms other than the modulation of inflammation. One potential mechanism could be an influence on vascular function, as recently studied by measuring the skin microvascular responses to iontophoretic administration of the endothelium-dependent vasodilator acetylcholine (63). Endothelial function was indeed significantly better in children who had been BF than in those who had received infant milk formula (63). Das et al. (15) proposed that cardiovascular benefits associated with BF could be partially mediated by long-chain PUFA in breast milk, through the serotonin-dopamine system or through the effects of insulin and insulin growth factor-I and -II on brain regulation. Interpretation of the biomarkers of cardiovascular risk assessed in our study have been criticized because of their association with acute phase reactivity (64).

Our study showed no association between BF, either exclusive or partial, in infancy and inflammatory status in adolescence in a large and well-described sample of European adolescents. Such an association cannot be excluded but might become apparent at a later age. Groups at high risk for cardiovascular disease, such as people who are overweight or obese, have a history of intrauterine growth retardation or prematurity, or have a family history of cardiovascular disease should be studied in future research in the context of the long-term effects of BF in infancy on later health. BF has many benefits beyond protection against inflammatory status later in life and such benefits outweigh any potential harm, so that BF remains the most suitable way of feeding infants.

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