Objective: To examine the association of exclusive breastfeeding (BF) duration on serum fibrinogen levels of children and adolescents from Estonia and Sweden, controlling for other potential confounding factors that could mediate in this relationship.

Design: Cross-sectional study.

Setting: Estonia and Sweden.

Participants: A total of 704 children (mean [SD] age, 9.5 [0.4] years) and 665 adolescents (15.5 [0.5] years).

Main Exposure: Exclusive BF duration was reported by the mother and categorized in the following 5 categories: never, less than 1 month, 1 to 3 months, more than 3 to 6 months, and more than 6 months.

Main Outcome Measures: Fasting fibrinogen level. Age, sex, pubertal status, country, adiposity (sum of 5 skin-fold thicknesses), total cholesterol and triglyceride levels, blood pressure, physical activity (accelerometry), birth weight, maternal education, body mass index, and age were considered confounders in the analyses.

Results: Longer duration of exclusive BF was associated with lower fibrinogen levels regardless of confounders (P < .001). Mean (SD) fibrinogen levels were lower in youth who were breastfed for more than 3 months (after adjusting for all confounders, P < .01) in children (2.55 [0.04] vs 2.77 [0.03] g/L), adolescents (2.59 [0.06] vs 2.72 [0.03] g/L), boys (2.47 [0.04] vs 2.73 [0.04] g/L), and girls (2.60 [0.03] vs 2.75 [0.02] g/L), compared with groups who were not breastfed. The results did not change substantially after further adjustment for birth weight and maternal educational level.

Conclusions: Exclusive BF is associated with less low-grade inflammation, as estimated by serum fibrinogen levels, in healthy children and adolescents. These findings give further support to the notion that early feeding patterns could program cardiovascular disease risk factors later in life.


Breastfeeding (BF) has been associated with a protective effect against cardiovascular disease (CVD) and obesity development later in life, but the evidence is inconsistent. Recent reviews have highlighted the need for further research controlling for relevant confounders influencing BF duration and health outcomes. Moreover, few studies in the literature identify subjects who were exclusively breastfed in early life and collect data on the duration of exclusive BF.

Previous studies showed that low-grade inflammation could be implicated in the development of CVD from early stages of life. Inflammatory markers are independent risk factors for coronary and vascular diseases. Increased serum fibrinogen levels have been associated with cardiovascular events. Studies investigating the influence of BF on low-grade inflammation markers are scarce and have contradictory results. Two studies reported lower levels of inflammatory markers in adults, and one reported them in preterm adolescents who were breastfed in infancy. In contrast, 2 studies did not find significant associations between BF and inflammatory marker levels in adults or in adolescents. Two of these 5 studies examined the influence of exclusive BF on inflammatory markers; they showed that BF with banked breast milk for 4 weeks was associated with lower levels of C-reactive protein (CRP) in adolescents born preterm and that BF for at least 1 month was related to lower fibrinogen and CRP levels in women.

Investigations with larger sample sizes and clear definition of exclusive BF are needed. Moreover, the optimal duration of exclusive BF and what should be recommended from a public health point of view are currently under debate. The aim of this study was to investigate the asso-
ciation of duration of exclusive BF with serum fibrinogen levels in children (aged 9-10 years) and adolescents (aged 15-16 years), controlling for potential confounding factors that could mediate in this relationship.

METHODS

SUBJECTS

The children and adolescents were participants in the Estonian (n=1050) and Swedish (n=319) parts of the European Youth Heart Study, a multicenter study examining the interactions among personal, environmental, and lifestyle influences on risk factors for future CVD. The study design, selection criteria, and sample calculations have been reported elsewhere. The study protocol was performed in accordance with the ethical standards laid down in the 1961 Declaration of Helsinki (as revised in 2000) and approved by the research ethics committees of the University of Tartu (No. 49/30-199); O¨ rebro County Council, Sweden (No. 690/98); and Huddinge University Hospital, Huddinge, Sweden (No. 474/98). Study procedures were explained to the participating youths and their parents or legal guardians; youths gave verbal assent and 1 parent or legal guardian provided written informed consent. Study procedures were explained to the participating youths and their parents or legal guardians; youths gave verbal assent and 1 parent or legal guardian provided written informed consent. The present study included a total of 704 children (mean [SD] age, 9.3 [0.4] years) and 665 adolescents (13.5 [0.5] years) with complete data on BF, fibrinogen levels, and body mass index (BMI). Subjects were apparently healthy, had no contraindications to any of the study procedures, and were not taking medications that might influence the results. No differences by BF prevalence and duration, maternal educational level, age, BMI, or sex distribution of the participants were found between youths with missing (n=189) and available data on exclusive BF (all P > .1).

NEONATAL DATA

Data on BF were collected by means of parental recall using a questionnaire. Mothers were asked to respond to the following 2 questions concerning BF at the time of the examination of the children:

1. Was your child fed completely on breast milk for any length of time, that is, without complementary bottle feedings?
2. If yes, for how long was your child breastfed? (categories provided for response were <1, ≥1 to 3, >3 to 6, and >6 months).

MEASUREMENTS

Blood samples were obtained by venipuncture after a 10-hour overnight fast. Serum fibrinogen levels were measured using commercially available kits (DakoCytomation, Glostrup, Denmark) and had sensitivities of 0.6 to 13.0 g/L and coefficients of variation of less than 4.8%.

Several variables potentially related to the relationship between BF and CVD risk factors and with the duration of exclusive BF were taken into account.18-20

Height, weight, and waist circumference were measured by standardized procedures, and BMI was calculated as weight in kilograms divided by height in meters squared. Overweight/obesity status was defined following the International Obesity Task Force that proposed sex- and age-adjusted BMI cutoff points.21 Skin-fold thicknesses were measured at the biceps, triceps, subscapular, suprailiac, and triceps surae areas. The sum of 5 skin-fold thicknesses and waist circumference were also used to estimate total and central body fat, respectively.

RESULTS

Among the 1369 children and adolescents included in the analyses, 83.0% had exclusive BF (Table 1); 19 partici-
pants had a mixed diet as infants (BF and bottle milk). Because the inclusion of mixed-diet subjects in the comparisons may dilute any potential advantageous or harmful effect, they were excluded from the analyses. Breastfeeding was more frequent ($P_{H.1021}=.001$) and of longer duration ($P_{H.1021}=0.002$) among Swedish than Estonian youths (Table 1).

Maternal and youths sociodemographic variables and study sample characteristics with available data on exclusive BF ($n=1350$) are presented in Table 2. There were no significant differences in age, pubertal status, sex distribution, age group (children or adolescents), total and central adiposity, overweight prevalence, maternal age at the child’s birth, physical activity, total cholesterol and triglyceride levels, diastolic blood pressure, and mean arterial blood pressure across BF duration categories. Lower systolic blood pressure was associated with longer duration of BF ($P_{H.1049}=.049$). Higher maternal educational level ($P_{H.1001}<.001$) and birth weight ($P_{H.1003}=.03$) were associated with longer duration of BF. Therefore, the analyses between BF duration and fibrinogen levels were additionally adjusted for these variables.

### Table 1. Patterns of Exclusive BF Duration in Swedish and Estonian Children and Adolescents

<table>
<thead>
<tr>
<th>Country, % of Participants</th>
<th>Children (n=531)</th>
<th>Adolescents (n=519)</th>
<th>Children (n=173)</th>
<th>Adolescents (n=146)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No BF</strong></td>
<td>16.0</td>
<td>21.6</td>
<td>11.6</td>
<td>11.0</td>
</tr>
<tr>
<td><strong>Mixed infant feeding (bottle and BF)</strong></td>
<td>1.9</td>
<td>1.5</td>
<td>0.0</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Duration of BF, mo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>11.5</td>
<td>12.9</td>
<td>8.2</td>
<td>8.3</td>
</tr>
<tr>
<td>1 to 3</td>
<td>46.9</td>
<td>36.4</td>
<td>20.3</td>
<td>26.9</td>
</tr>
<tr>
<td>&gt;3 to 6</td>
<td>14.5</td>
<td>17.2</td>
<td>40.7</td>
<td>35.2</td>
</tr>
<tr>
<td>&gt;6</td>
<td>9.2</td>
<td>10.4</td>
<td>19.2</td>
<td>17.9</td>
</tr>
</tbody>
</table>

Abbreviation: BF, breastfeeding.

### Table 2. Descriptive Characteristics of Children and Adolescents Among Exclusive BF Duration Categories

<table>
<thead>
<tr>
<th>Duration of BF, mo</th>
<th>Never (n=233)</th>
<th>&lt;1 (n=154)</th>
<th>1 to 3 (n=512)</th>
<th>&gt;3 to 6 (n=289)</th>
<th>&gt;6 (n=162)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>12.8 (3.0)</td>
<td>12.6 (3.0)</td>
<td>12.2 (3.0)</td>
<td>12.4 (3.0)</td>
<td>12.4 (3.0)</td>
<td>.10</td>
</tr>
<tr>
<td>Children, %</td>
<td>46.3</td>
<td>48.7</td>
<td>55.5</td>
<td>51.2</td>
<td>50.6</td>
<td>.11</td>
</tr>
<tr>
<td>Puberty stage, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.31</td>
</tr>
<tr>
<td>1</td>
<td>34.1</td>
<td>41.0</td>
<td>41.6</td>
<td>41.2</td>
<td>41.6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>11.6</td>
<td>9.0</td>
<td>12.0</td>
<td>11.9</td>
<td>10.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7.3</td>
<td>7.0</td>
<td>8.3</td>
<td>7.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>20.4</td>
<td>20.5</td>
<td>17.3</td>
<td>14.8</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>&gt;5</td>
<td>26.5</td>
<td>22.5</td>
<td>24.2</td>
<td>28.4</td>
<td>27.3</td>
<td></td>
</tr>
<tr>
<td>Female sex, %</td>
<td>61.3</td>
<td>54.5</td>
<td>51.4</td>
<td>55.4</td>
<td>49.4</td>
<td>.09</td>
</tr>
<tr>
<td>Sum of 5 skin-fold thicknesses, mm</td>
<td>47.5 (21.2)</td>
<td>44.2 (20.0)</td>
<td>44.2 (19.4)</td>
<td>44.9 (18.8)</td>
<td>44.5 (18.9)</td>
<td>.29</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>63.0 (8.2)</td>
<td>63.0 (8.3)</td>
<td>62.3 (8.0)</td>
<td>63.2 (7.8)</td>
<td>63.9 (8.5)</td>
<td>.24</td>
</tr>
<tr>
<td>Overweight/obese, %</td>
<td>22.7</td>
<td>15.6</td>
<td>18.4</td>
<td>18.8</td>
<td>18.5</td>
<td>.43</td>
</tr>
<tr>
<td>Maternal educational level below university, %</td>
<td>73.2</td>
<td>77.6</td>
<td>71.5</td>
<td>63.5</td>
<td>46.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Maternal age at childbirth, y</td>
<td>29.8 (4.9)</td>
<td>28.7 (5.1)</td>
<td>29.2 (5.7)</td>
<td>29.7 (4.4)</td>
<td>29.7 (4.9)</td>
<td>.87</td>
</tr>
<tr>
<td>Physical activity, counts/min</td>
<td>599 (220)</td>
<td>608 (232)</td>
<td>645 (281)</td>
<td>635 (260)</td>
<td>608 (251)</td>
<td>.34</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>3422 (700)</td>
<td>3461 (596)</td>
<td>3555 (555)</td>
<td>3540 (571)</td>
<td>3565 (544)</td>
<td>.03</td>
</tr>
<tr>
<td>Mean triglyceride level, mg/dL</td>
<td>72</td>
<td>67</td>
<td>68</td>
<td>69</td>
<td>70</td>
<td>.17</td>
</tr>
<tr>
<td>Mean total cholesterol level, mg/dL</td>
<td>168</td>
<td>168</td>
<td>167</td>
<td>166</td>
<td>165</td>
<td>.62</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>109.8 (10.7)</td>
<td>108.6 (13.3)</td>
<td>107.2 (11.5)</td>
<td>107.7 (10.7)</td>
<td>108.6 (12.5)</td>
<td>.049</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>63.0 (6.8)</td>
<td>61.4 (7.9)</td>
<td>61.7 (7.5)</td>
<td>62.4 (6.6)</td>
<td>61.9 (6.5)</td>
<td>.11</td>
</tr>
<tr>
<td>Arterial pressure</td>
<td>79.6 (7.3)</td>
<td>77.1 (8.9)</td>
<td>76.9 (8.1)</td>
<td>77.5 (7.1)</td>
<td>77.5 (7.5)</td>
<td>.06</td>
</tr>
</tbody>
</table>

Abbreviation: BF, breastfeeding.

SI conversion factor: To convert cholesterol to millimoles per liter, multiply by 0.0259; triglycerides to millimoles per liter, multiply by 0.0113.

a Data are expressed as mean (SD) unless otherwise indicated.

b Percentages have been rounded and might not total 100.

c Analysis was performed on log-transformed data, but nontransformed data are presented in the Table.

d Physical activity was measured using an activity monitor.

e Arterial pressure was calculated as diastolic blood pressure + [0.333 × (systolic blood pressure - diastolic blood pressure)].

**EXCLUSIVE BF DURATION AND FIBRINOGEN LEVELS**

Children and adolescents who had ever been exclusively breastfed had lower fibrinogen levels than those...
who had never been breastfed (2.61 [0.48] vs 2.76 [0.56] g/L; mean difference, 0.15 [95% CI, 0.09-0.22] g/L after adjusting for age, sex, pubertal status, and country. The results did not differ after further adjustment for adiposity, mean arterial pressure, total cholesterol and triglyceride levels, and physical activity (mean difference with adjustments, 0.11 [95% CI, 0.03-0.19] g/L).

Longer duration of BF was associated with lower fibrinogen levels regardless of age, pubertal status, sex, country, physical activity, total adiposity, mean arterial pressure, total cholesterol and triglyceride levels (P < .001; Figure, A), and central adiposity (P < .001). The association persisted after further controlling for birth weight and maternal educational levels (P < .001). There was no significant interaction effect between country and BF with regard to fibrinogen levels. Thus, we observed similar trends in youths from both countries, although the results were statistically significant only in Estonian participants (P = .008 and P = .10, for Estonian and Swedish participants, respectively), probably owing to the smaller Swedish sample size. The results were consistent in children and adolescents and in girls and boys (Figure, B and C). These results did not substantially differ (P = .01) when the analyses were restricted to nonsmoker participants (91.8% of the sample; 112 were smokers [41 female and 71 male]).

We observed that fibrinogen levels were significantly lower in participants who were breastfed for at least 3 months than in subjects who were never breastfed in infancy (Figure). Indeed, fibrinogen levels differed across BF categories (ie, ≤3 vs >3 months), in children (2.55 [0.04] vs 2.77 [0.03] g/L, mean difference, 0.18 [95% CI, 0.01-0.27] g/L; P < .001, adjusted for age, pubertal status, sex, country, adiposity, mean arterial pressure, total cholesterol and triglyceride levels, and physical activity), adolescents (1.60 [0.03] vs 2.75 [0.02] g/L, mean difference, 0.12 [95% CI, 0.03-0.21] g/L; P = .01, adjusted for all covariates), boys (mean difference, 0.16 [95% CI, 0.01-0.25] g/L; P < .001, adjusted for all covariates), or girls (mean difference, 0.15 [95% CI, 0.05-0.24] g/L; P = .003, adjusted for all covariates). The results did not substantially change after further adjustment for birth weight and maternal educational level or when the analyses were adjusted for central adiposity instead of total adiposity (data not shown).

**COMMENT**

The present study shows that longer exclusive BF in infancy is associated with lower serum fibrinogen levels in Estonian and Swedish children and adolescents, independently of potential confounders such as adiposity and physical activity, which have been associated with lower inflammatory markers in youths,25-27 and regardless of sociodemographic variables affecting BF duration, such as birth weight and maternal educational levels. We also observed that differences in fibrinogen concentrations became significant when children and adolescents were breastfed for at least 3 months.

Several studies have examined the relationship between BF and low-grade inflammation markers in adolescents and adults; however, as far as we are aware, this is the first study examining the programming effect of early BF on low-grade inflammation in a relatively large sample of children and providing data about the effect of duration of exclusive BF. Our results confirm the results of Singhal et al.,15 who found lower levels of low-grade inflammation, as estimated by CRP levels, in 216 preterm-born adolescents who were breastfed for 4 weeks than in those fed with preterm formula. We also extend this observation to a larger and nonspecific population (ie, nonslected according to their gestational age) of apparently healthy children and adolescents. In the study by Vérier et al.,16 CRP levels were only marginally associated (P = .1) with BF duration after adjustment for CVD risk factors,
whereas our results persisted after controlling for total cholesterol and triglyceride levels and blood pressure. This discrepancy in the results could be a result of the sample size. Indeed, they found that adolescents who had been breastfed for at least 6 months (n = 116) showed lower CRP concentrations than those who had not been breastfed (n = 90). The study by Williams et al conducted in a group of 375 women aged 26 years also showed that any BF for at least 6 months was associated with lower CRP concentrations, in agreement with Vérier et al. The results provided by 2 previous studies conducted in older adults were controversial. Likewise, in a large study performed in adults (9377 subjects, aged 44-45 years), Rudnicka et al found that any BF for at least 1 month was associated with lower CRP and fibrinogen levels in women, whereas Martin et al did not find any relationship between BF and inflammation in another study of adult men (n = 1062 men), although the authors acknowledged that selection and recall bias (more than 50 years since birth) may have affected their results.

Findings of our study suggest that the infant feeding method permanently affects an important inflammatory marker associated with atherosclerosis and CVD later in life. We found that exclusive BF (ever BF) is associated with a reduction of approximately 4.5% in serum fibrinogen levels. Moreover, the difference between youths never breastfed and those breastfed increased up to 7.3% (7.6% in children and 7.0% in adolescents) when exclusive BF duration was longer than 3 months. Although it is unknown what cutoff point constitutes a clinically high level, fibrinogen concentration is an established CVD risk factor. Our results, together with previous epidemiological and experimental findings, suggest that exclusive BF in infancy for at least 3 months has beneficial effects on cardiovascular health later in life. To our knowledge, this is the first report examining the association between duration of exclusive BF and fibrinogen level, which hampers comparisons. Indeed, the exposure (ie, exclusive or any BF and duration of BF) of the 5 studies examining the relationship between BF and inflammation was different. Two studies reported the results of exclusive BF on inflammatory markers. One of them compared bottle feeding with exclusive BF for the first 4 weeks. Two compared BF of any duration with bottle feeding, one compared any BF with no BF, and the fifth examined the influence of BF for at least 1 month compared with no BF. None of them reported data on a possible dose-response influence of BF on low-grade inflammation markers.

The optimal duration of exclusive BF is one of the most debated areas in childhood nutrition. We did not find any significant difference in fibrinogen levels between BF for 3 to 6 months and for more than 6 months. This observation is in accordance with other studies examining the association of BF duration with CVD risk factors. Lavor et al showed the lowest values of blood systolic pressure in youths who were exclusively breastfed for 3 to 6 months, without any significant differences between being exclusively breastfed for 3 to 6 months or for more than 6 months. Evelein et al identified the same period of exclusive BF duration (from 3-6 months) as the most effective to increase the carotid intima-media thickness in children 5 years of age. O’Tierney et al also reported that subjects who were breastfed for less than 2 or more than 8 months had higher adiposity than did those who were breastfed for 2 to 8 months. Nevertheless, these findings should be taken with caution owing to the categorization of duration of BF in 4 groups instead of as a continuous variable. Further investigations with detailed information of infant feeding—preferably recorded at the time of feeding—and with more biological markers of inflammation are needed, such as CRP level.

The potential mechanisms involved in metabolic programming in the context of the developmental origins of health and disease of low-grade inflammation need further investigation. We have previously shown an association between low birth weight and higher levels of chronic inflammation in Swedish children and adolescents. However, biological mechanisms acting during pregnancy and in the first steps of postnatal growth are unclear; stress responses programmed during the critical periods of prenatal and postnatal development could lead to inflammatory responses still later in childhood and adolescence.

Our study has several strengths. The relatively large sample size and the availability of potential confounders could mediate in the association between BF and fibrinogen. Moreover, the opportunity to examine the association of exclusive BF instead of any BF, in which the associations could be diluted, should be highlighted. Maternal recall of BF approximately 9 years later in children and 15 years later in adolescents and the recording of the duration of BF in months instead of in weeks must be acknowledged as limitations of the present report. In addition, although all the participants were apparently healthy, we cannot exclude the possibility that some children had recent infections that could increase fibrinogen levels. Moreover, we have no information about the use of oral contraceptives in female adolescents (27.1% of the sample). Finally, previous studies have reported diurnal and seasonal variations in fibrinogen that may be sources of bias in the analyses of epidemiological studies. However, in our study, all samples were taken early in the morning in the fasting state to avoid the effect of diurnal variations. Furthermore, the biological relevance of fibrinogen seasonality is uncertain because it was not found in young and early-middle-aged adults, suggesting a potential role of aging or physical activity.

In conclusion, besides the previously reported benefits of BF in the short- and long-term health of individuals, exclusive BF in infancy is associated with lower fibrinogen levels in children and adolescents. Therefore, part of the association of BF with later atherosclerosis and cardiovascular risk could be mediated by lower inflammatory protein concentrations. Exclusive BF should be advocated, when possible, as the preferred method of feeding infants for at least the first 3 months of life.

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