Breastfeeding and risk of inflammatory bowel disease: a systematic review with meta-analysis[1–3]

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ABSTRACT

Background: It has long been believed that breastfeeding provides protection against ulcerative colitis and Crohn disease. Studies designated to test this hypothesis were conducted without reaching conclusive results.

Objective: The aim of this meta-analysis was to examine the role of breastfeeding in preventing inflammatory bowel disease and to summarize the evidence gathered about this subject.

Design: A meta-analysis was performed on 17 relevant articles that were found by using MEDLINE, EMBASE, the Internet, and articles' references. The publications were fully reviewed and divided, on the basis of their quality, into 3 groups.

Results: Studies showed heterogeneous results. The pooled odds ratios of all the 17 reviewed studies, calculated according to the random-effects model, were 0.67 (95% CI: 0.52, 0.86) for Crohn disease and 0.77 (0.61, 0.96) for ulcerative colitis. However, only 4 studies for Crohn disease and 4 for ulcerative colitis were eventually included in the highest quality group. In this group, the pooled odds ratio was 0.45 (0.26, 0.79) for Crohn disease and 0.56 (0.38, 0.81) for ulcerative colitis.

Conclusions: The results of this meta-analysis support the hypothesis that breastfeeding is associated with lower risks of Crohn disease and ulcerative colitis. However, because only a few studies were graded to be of high quality, we suggest that further research, conducted with good methodology and large sample sizes, should be carried out to strengthen the validity of these observations. *Am J Clin Nutr* 2004;80:1342–52.

KEY WORDS   Crohn disease, ulcerative colitis, breastfeeding, meta-analysis, epidemiology

INTRODUCTION

Despite intensive investigation into the cause and pathogenesis of inflammatory bowel disease (IBD), its pathogenic mechanism has yet to be elucidated. Several studies indicate that a genetic basis exists for these diseases and showed a correlation between disease prevalence and the presence of specific genomic markers (1, 2). Whatever role genetic loci might play in conferring susceptibility to IBD, studies of identical twins [in which only 45% of identical twin pairs are concordant for Crohn disease (CD)] suggest that additional environmental factors are necessary for the development of this disease (3).

To determine which environmental factors contribute to the development of CD or ulcerative colitis (UC), numerous epidemiologic studies were performed (4–7). Factors such as smoking

(8) and use of oral contraceptives (9) were meta-analyzed to determine their role on the risk of IBD development.

In this meta-analysis, we evaluate another factor, ie, the effect of breastfeeding, on the later development of UC and CD. The reasoning is 3-fold. First, breastfeeding protects against many immune-mediated diseases such as bronchial asthma (10), atopic dermatitis (11), allergic rhinitis (12), and type 1 diabetes mellitus (13). This effect is attributed to the immunomodulatory properties of human milk. From here we hypothesized that if the immunomodulatory effect of breastfeeding offers protection against these diseases, it is plausible to assume similar protection with regard to UC and CD. Second, the infant is exposed to human milk while developing an immune system, which seems to be important in procuring oral tolerance to specific microflora and food antigens, which can play a role in the pathogenesis of IBD (14). Third, breast-milk feeding was shown to limit the development of colitis in mice deficient for interleukin 10. This finding was explained by the change of intestinal flora of the developing mice from pathogenic bacteria to nonadherent bacteria as a result of oligosaccharides found in the milk that stimulate *Bifidobacterium* and *Lactobacillus* growth (15). A change in proinflammatory cytokine secretion can also be offered as an explanation (16).

Yet, most of the findings about the beneficial effect of breastfeeding derive from epidemiologic studies. Indeed, some studies found breastfeeding to be protective against UC or CD (17–22). However, most of the studies failed to achieve statistically significant results or found no association at all (4, 5, 23–30). Meta-analyses of observational studies present particular challenges because of inherent biases and differences in study designs (31). Thus, this meta-analysis, which is reported here according to the "proposal for reporting" published previously by Stroup et al (32), does not presume to provide a precise estimate of the association between breastfeeding and IBD but rather attempts to

either support or weaken this hypothesis and to summarize the evidence that was gathered about this subject.

SUBJECTS AND METHODS

Search strategy

A meta-analysis was performed on the basis of a computerized search of English-written epidemiologic (case-control or cohort) studies of the association between breastfeeding and UC or CD listed in the MEDLINE (National Library of Medicine, Bethesda, MD) and EMBASE (Elsevier Publishers BV, Amsterdam) data banks before November 2003. Specifically, a literature search was performed (by the investigators with the aid of a professional librarian) by using the index terms *ulcerative colitis*, *Crohn disease*, *inflammatory bowel disease*, *bottle-feeding*, *breastfeeding*, *infant nutrition*, *perinatal*, and *milk* in various combinations.

From the abstracts identified in the database search, 14 described relevant epidemiologic studies and were selected for full review (4, 5, 17–26, 28, 29). By reviewing the references of these articles, 2 additional studies were discovered (27, 30). An Internet search was conducted as well by using the same terms used in the database search to locate published studies not registered in MEDLINE or EMBASE. This search recovered one additional study (33). Thus, a total of 17 studies were fully reviewed in this meta-analysis.

We selected all studies in which the primary or secondary goal was to evaluate the association between breastfeeding and UC or CD as separate entities. These articles were independently reviewed by the authors (EK and RVC) by using a standardized report form. The articles were graded according to predefined guidelines that will be further detailed. Discrepancies were resolved in conferences.

A primary prerequisite for the inclusion of studies in the meta-analysis was the presence of a control group, which could be formed by population controls, by hospital inpatients, or by outpatients who did not suffer from IBD or other chronic diseases that might be related to lack of breastfeeding. Studies, in which the control subjects were recruited to the study by the case subjects, with no supervision of the investigators or coinvestigators, were considered to be of lower quality, because this recruitment method could inflict a serious selection bias. To further deal with the problem of selection bias, we categorized the studies according to the percentage of subjects willing to participate from the total number of subjects approached by the investigators (response rate); ie, articles in which the investigators did not detail response rates or recorded response rates of <80% in either the case or the control subjects were ranked as having a lower quality.

Studies were categorized according to the age of diagnosis, from birth to adolescence (0–18 y) or adults (>18 y). To decrease information bias, studies of adults, which did not specifically note that the classification of breastfeeding was based on information collected from parents or another older relatives of the participating subjects, were classified as low quality. This classification was not a requirement in pediatric studies (provided that data were collected during childhood), because it was assumed that the information was obtained from a parent or an older relative. No restrictions were imposed on the method in which this information was obtained (mail, interview, or clinical files).

No restrictions were imposed on the method of diagnosis of CD and UC. As long as the diagnosis was confirmed by a physician, we assumed that well-trained specialists diagnosed the cases. Otherwise, the study was assigned to the low quality group.

Breastfeeding was defined as either exclusive or nonexclusive breastfeeding for any given duration. Accordingly, *no breastfeeding* was defined as nonexclusive or exclusive bottle-feeding from birth. When odds ratios (ORs) were calculated for both definitions, we used the OR for "not exclusively breastfed" for any duration compared with "exclusively bottle-fed from birth" for the calculation of the pooled estimate. Duration of breastfeeding was sought and documented.

We did not exclude studies in which the investigators stated that the correlation between breastfeeding and CD or UC was insignificant and, therefore, presented neither OR nor crude data. Instead, the OR was estimated to be 1, and the CI was calculated by assuming participation of all subjects in the study, and by arbitrarily assigning them a rate of 20% bottle-feeding. In this manner we maintained a conservative attitude in which it was more difficult to spuriously reject the null hypothesis of no relation between breastfeeding and IBD.

To sum up this section, studies were graded for quality levels as follows. For grade 1 (best quality), case and control subjects were recruited by the investigators or coinvestigators. Diagnosis was always confirmed by a physician, and breastfeeding information was always confirmed by patients' mothers or other older close relative (as previously mentioned, this was not a requirement in pediatric studies). Response rate is mentioned in the article and is ≥80% for both case and control subjects. Grade 2 was the same as grade 1, except that the response rate is not mentioned or is <80%. For grade 3 (lowest quality), either breastfeeding information was not provided by the mother or a close relative of the patient, diagnosis was not confirmed by a physician, or control subjects were recruited to the study by the patients.

Statistical analysis

The pooled OR and its confidence limits were calculated by using the DerSimonian and Laird method (34), which is based on the random-effects model. The fixed-effects model–based OR, calculated as previously described by Greenland (35), is also presented. In both methods, the weight of each study depends on the inverse of the variance of log OR, which is estimated by the 95% CI of each study.

Heterogeneity of the studies was calculated with the following formula:

$$\chi^2_{\text{heterogeneity}} = \sum w_i \times [\ln(OR_i) - \ln(OR)]^2 \qquad (1)$$

The df for the chi-square test was defined as the number of studies minus one [w_i represents the weight (calculated by the inverse of the variance) of each study].

In studies in which both crude and adjusted OR were reported, we included the adjusted OR in our calculation of the pooled estimate. If no single adjusted OR was presented, we included the crude OR. If no OR was presented in a given study, we calculated it and its 95% CI according to the raw data presented in the article.

Publication bias was investigated by funnel plots and by the regression asymmetry test for skewed funnel plot introduced by Egger et al (36). A low *P* value in this test suggests the possibility of a publication bias.

Data analysis was performed by using PEPI 4.0 and COMPARE2 version 1.25 statistical package (Sagebrush Press, Salt Lake City) (37). A P value < 0.05 was considered to be statistically significant.

RESULTS

Of the 17 studies that were included in the meta-analysis presented here, 11 investigated both UC and CD, 3 investigated UC alone, and 3 investigated CD alone. Together a total of 2577 patients with UC and 3551 control subjects and 3190 patients with CD and 4026 control subjects were studied. The studies are summarized for UC (**Table 1**) and for CD (**Table 2**). Four studies did not present the exact findings about breastfeeding (4, 23, 27, 30). Instead, they merely claimed that the OR was close to unity. The OR in those studies was estimated as 1, and the CI was calculated as described in "Subjects and Methods." An exception was made for the study of Gilat et al (4), because this researcher calculated the OR from the number of discordant pairs of each case subject and the matched control subject. Thus, the CI for that study was calculated with half of the control subjects mentioned in the article (302 pairs of control and case subjects for CD and 197 pairs for UC).

Studies were graded in accordance with the criteria mentioned in Subjects and Methods (**Table 3**). Overall, only 4 studies (consisting of 397 patients with UC and 766 control subjects and 583 patients with CD and 876 control subjects) were included in the highest quality groups for either CD (18, 20, 21, 24) or UC (17, 21, 24, 26). The pooled ORs and 95% CIs (calculated according to the random-effects model) for these studies were 0.56 (95% CI: 0.38, 0.81) for UC and 0.45 (95% CI: 0.26, 0.79) for CD. ORs for the 8 UC studies and the 7 CD studies graded in quality groups 1 and 2 were 0.61 (95% CI: 0.46, 0.83) and 0.55 (95% CI: 0.34, 0.87), respectively. When all studies were included in the pooled estimate, the random-effects model OR was 0.77 (95% CI: 0.61, 0.96) for UC and 0.67 (95% CI: 0.52, 0.86) for CD (**Table 4**, **Figures 1** and **2**). Thus, the protective effect of breastfeeding against both diseases remained statistically significant for all calculated pooled ORs. However, the results for both diseases appeared to be heterogeneous, primarily after adding the studies of lower quality ($P_{heterogeneity}$ < 0.001 for CD and $P_{heterogeneity}$ = 0.002 for UC). Heterogeneity was further explored by dividing the studies according to various characteristics related to population differences, exposure definition, and methodologic issues and by calculating summary estimates of the OR for the association of UC and CD with breastfeeding for each group (**Table 5**).

Exploration of the possibility for publication bias by funnel plots (**Figures 3** and **4**) indicated a possible publication bias in the studies for CD. P value for a skewed funnel plot, calculated by the regression asymmetry test, was 0.23 for the UC studies and 0.003 for CD studies.

DISCUSSION

The overall pooled OR of this meta-analysis demonstrates that breastfeeding has a statistically significant protective role against UC and an even greater role against CD. Because exclusion of studies is subject to criticism as a result of influence of former beliefs, we did not exclude studies but rather calculated separate pooled ORs for the best quality studies, for best and intermediate quality studies, and for all studies. The protective

effect of breastfeeding against both diseases remained statistically significant for all calculated pooled ORs.

The test for heterogeneity, however, was statistically significant for both UC and CD. This finding can be partly explained by differences in the case subjects' age (children and adolescents compared with adults), control subjects characteristics (hospital based compared with population based), matching variables, and the exact definition of breastfeeding (Table 5). Heterogeneity of the studies can also be attributed to the differences in the quality of the studies, because the results become more heterogeneous when studies with lower quality are included. These differences can be due to biased results of these studies. Of special concern is the study of Thompson et al (28). That study incorporated hundreds of CD and UC case subjects in its investigation. Thus, despite methodologic problems, which made it highly prone to various biases, it had the highest influence on the pooled OR and the heterogeneity of the studies.

All but 2 studies recovered in this meta-analysis were retrospective case-control studies. That type of study constitutes a drawback because case-control studies are subject to misclassification as a result of recall bias and to selection bias. It is, however, difficult to conduct a prospective study that tests the relation between IBD and breastfeeding, because the lag between breastfeeding and the development of IBD is substantial. In 2 of the studies (24, 29), however, data about breastfeeding was collected from medical records and, thus, did not rely on the recall of mothers. Nevertheless, these data were recorded merely a few days after labor. The implications of this data collection will be further discussed later in this article.

Selection bias is potentially present in all of the reviewed studies, because no study described a comparison between subjects participating in the study and subjects excluded or not willing to participate. We set a low rank to studies in which the case subjects were instructed to obtain replies to the control subject's questionnaire by themselves, because the process of selecting and questioning the control subjects by the case subjects without the supervision of the investigators is, in our opinion, highly prone to selection bias. To further minimize the possibility of selection bias, we calculated a distinct pooled OR for the studies in which response rate was specified and was ≥80%.

Another potential source of bias is related to imprecise recall of breastfeeding. Thus, studies in which information about breastfeeding was not provided by the mother of the patient or an older close relative were assigned to the lowest quality group. Data provided by mothers, theoretically, could also be prone to recall bias, when one considers the prolonged lag time elapsing from infancy to development of the disease. However, we tend to think that this kind of bias was not an important problem in those studies. Our thought is supported by a study conducted by Launer et al (38) that demonstrated a high accuracy in the recall of breastfeeding duration at 18 mo after birth. Although in the reviewed studies, breastfeeding practices were inquired years after birth, the information that the mothers were asked to obtain was simple (breastfeeding, yes or no); hence, we believe it was accurate. Furthermore, our thoughts are supported by Bergstrand and Hellers (18), who mentioned in their study that "most living mothers were remarkably exact in their information regarding breastfeeding." Nevertheless, duration of breastfeeding was not documented in most of the studies. In light of the dose–response effect found for both CD and UC by Rigas et al (21) and for CD

TABLE 1
Case-control studies testing the association between breastfeeding and ulcerative colitis[1]

Authors, year of publication, place	Quality group	Sample size		Matching (beyond age and sex)	Age (diagnosis)	Response rate	Breastfeeding definition	Duration of breastfeeding	Crude OR (95% CI)	Adjusted OR (95% CI)
		Cases	Controls							
Acheson and Truelove, 1961 (17), United Kingdom (Oxford)	1	101[2]	98[2] hospital patients	No	Unknown	Cases, 87%; controls, 85%	Exclusive	2 wk	0.34 (0.17, 0.68)[3]	Age (at interview) adjusted: 0.38 (0.2, 0.7); residence region adjusted: 0.4 (0.2, 0.7); rank in family adjusted: 0.39 (0.21, 0.71); social class adjusted: 0.35 (0.18, 0.66)[4]
Ekbom et al, 1990 (24), Sweden (Uppsala)	1	164	328 population based (infant born to the first mother who was admitted to the hospital maternity ward after the case's mother)	Yes; by time of birth, mother's number of previous deliveries, and age	Median age (of patients with ulcerative colitis and Crohn disease): 25 y	Irrelevant (data reviewed from medical records)	Exclusive	Unknown (mean time of data availability: 10 d)	0.8 (0.5, 1.4)	NA
Koletzko et al, 1991 (26), Canada (Toronto)	1	93	138 unaffected siblings	Yes; inherent in control selection	Mostly children	92%	No exact definition	No exact definition	NA	0.59 (0.27, 1.3) adjusted by conditional logistic regression for sex and for having diarrheal disease during infancy
Rigas et al, 1993 (21), United States (New York)	1	39	202 hospital controls having other gastrointestinal problems	No	Children	Irrelevant (data reviewed from medical records)	No exact definition	No exact definition	0.5 (0.25, 1.01)[3,5]	0.2–0.7 (depends on number of months of breast-feeding; adjusted for sex, age, race, birthplace, sibship size, birth order, and maternal age; pooled adjusted OR for all breastfeeding durations: 0.56 (0.3, 1.03)[4]
Whorwell et al, 1979 (22), United Kingdom (Southampton)	2	51	102 population and healthy hospital controls	No	Unknown	Unknown	Exclusive	No exact definition	0.32 (0.14, 0.75)[3]	NA
Gilat et al, 1987 (4), 9 countries (Europe, North America, Mediterranean)	2	197	197[6] population and hospital controls	Yes; by health center	Mostly children	Unknown	No exact definition	No exact definition	1 (0.61, 1.64)[7]	NA
Kono et al, 1994 (23), Japan	2	84	107 hospital controls free of cancer, peptic ulcer, and autoimmune and chronic bowel disease	Yes; by inpatient status	Children and adults	Unknown	No exact definition	No exact definition	1 (0.49, 2)[7]	NA

(Continued)

TABLE 1 (*Continued*)

Authors, year of publication, place	Quality group	Sample size — Cases	Sample size — Controls	Matching (beyond age and sex)	Age (diagnosis)	Response rate	Breastfeeding definition	Duration of breastfeeding	Crude OR (95% CI)	Adjusted OR (95% CI)
Urashima et al, 1999 (33), Japan	2	133	266 healthy persons or outpatients (excluding chronic diseases)	Yes[8]; by block of birth	Children	48.2%	Nonexclusive breastfeeding	No exact definition	0.53 (0.31, 0.89)	NA
							Exclusive breastfeeding	No exact definition	0.66 (0.4, 1.1)	
Corrao et al, 1998 (19), Italy	3	594	594 inpatients and outpatients with acute diseases	No	Mostly adults	Cases, 95%; controls, 94%	No exact definition	No exact definition	0.69 (0.5, 0.94)[3]	0.67 (0.48, 0.91) adjusted by conditional logistic regression for sex and for smoking status and use of oral contraceptives
Thompson et al, 2000 (29), United Kingdom	3	27	216 population based nested in 2 cohorts	Yes[8]; by social class	Unknown	Irrelevant (data reviewed from medical records)	No exact definition	No exact definition	2.76 (0.86, 8.81)	NA
Persson et al, 1993 (27), Sweden (Stockholm)	3	145	305 population-based controls	No	Children and adults	Cases, 80%; controls, 78%	No exact definition	2 mo	1 (0.61, 1.64)[7]	NA
Wurzelmann et al, 1994 (30), United States (North Carolina)	3	181	141 neighbors of the same sex, race, and age of patients	Yes[8]; inherent in control selection	Mostly adults	Cases, 73%; controls, 80%	No exact definition	No exact definition	1 (0.58, 1.73)[7]	NA
Klein et al, 1998 (5), Israel (Tel-Aviv)	3	55	144 population and hospital outpatient controls	Yes[8]; by country of origin and residential neighborhood	Children and adults	Unknown	No exact definition	1 mo	0.81 (0.39, 1.71)[9]	NA
Thompson et al, 1995 (28), United Kingdom	3	713	713 friends or neighbors of the same sex, ethnic origin, and age	Yes; by ethnic origin	Mostly adults	21%	No exact definition	No exact definition	1.16 (0.9, 1.5)	NA

[1] Studies in quality groups 1 and 2 are organized according to year of publication. Studies in quality group 3 are organized according to decreasing quality. OR, odds ratio; NA, not available.

[2] Analysis included merely cases and controls for whom history regarding breastfeeding status was confirmed.

[3] OR and 95% CI were calculated according to the data presented in the article.

[4] For all ORs, ORs and 95% CIs adjusted for the specified variable were calculated by using the Mantel-Haenszel method or the fixed-effects model described by Greenland (35) according to the data presented in the article.

[5] Negative association trend between duration of breastfeeding and risk of ulcerative colitis.

[6] Although 394 controls were included in the study, only half of them (197) were included in the matched analysis.

[7] The exact OR was not mentioned. Because the article indicated that no significant difference was found, we assumed the OR to be ≈1. The CI was calculated according to the sample size by assuming an exposure rate of 20% in both groups.

[8] Matching was not treated statistically in analysis.

[9] The OR and 95% CI were calculated according to the data presented in the article, assuming that all patients and controls provided information regarding breastfeeding.

TABLE 2
Case-control studies testing the association between breastfeeding and Crohn disease[1]

| Authors, year of publication, place | Quality group | Sample size | | Matching (beyond age and sex) | Age (diagnosis) | Response rate | Breastfeeding definition | Minimal duration of breastfeeding | Crude OR (95% CI)[3,4] | Adjusted OR (95% CI) |
		Cases	Controls							
Bergstrand and Hellers, 1983 (18), Sweden (Stockholm)	1	308	308 population based	Yes[2]; by area of birth	Children and adults	93%	No exact definition	1 mo	0.28 (0.14, 0.56)[3,4]	NA
Koletzko et al, 1989 (20), Canada (Toronto)	1	114	180 unaffected siblings	Yes; inherent in control selection	Mostly children	88%	No exact definition	No exact definition	0.28 (0.11, 0.71)	0.26 (0.11, 0.67) adjusted by conditional logistic regression for having diarrheal disease during infancy
Ekbom et al, 1990 (24), Sweden (Uppsala)	1	93	186 population based (infant born to the first mother who was admitted to the hospital maternity ward after the case's mother)	Yes; by time of birth, mother's number of previous deliveries, and age	Median age (of patients with ulcerative colitis and Crohn disease): 25 y	Irrelevant (data reviewed from medical records)	Exclusive	Unknown (mean time of data availability: 10 d)	1 (0.5, 2.2)	NA
Rigas et al, 1993 (21), United States (New York)	1	68	202 hospital controls having other gastrointestinal problems	No	Children	Irrelevant (data reviewed from medical records)	No exact definition	No exact definition	0.48 (0.27, 0.85)[3,4]	0.1–0.7 (depends on number of months of breastfeeding); adjusted for sex, age, race, birthplace, sibship size, birth order, and maternal age; pooled adjusted OR for all breastfeeding durations: 0.58 (0.32, 1.07)[5]
Whorwell et al, 1979 (22), United Kingdom (Southampton)	2	57	114 population and healthy hospital controls	No	Unknown	Not clear	Exclusive breastfeeding	No exact definition	1 (0.45, 2.23)[4]	NA
Gilat et al, 1987 (4), 9 countries (Europe, North America, Mediterranean)	2	302	302[6] hospital controls	Yes; by health center	Mostly children	Unknown	No exact definition	No exact definition	1 (0.67, 1.49)[7]	NA
Urashima et al, 1999 (33), Japan	2	42	126 healthy persons or outpatients (excluding chronic diseases)	Yes[2]; by block of birth	Children	44.7%	Nonexclusive breastfeeding	No exact definition	0.3 (0.13, 0.7)	NA
							Exclusive breastfeeding	No exact definition	0.46 (0.19, 1.1)	
Corrao et al, 1998 (19), Italy	3	225	225 inpatients and outpatients with acute diseases	No	Mostly adults	Cases, 97%; controls, 94%	No exact definition	No exact definition	0.67 (0.4, 1.1)[4]	0.53 (0.3, 0.91) adjusted by conditional logistic regression for sex and for smoking status and use of oral contraceptives

(Continued)

TABLE 2 (*Continued*)

Authors, year of publication, place	Quality group	Sample size		Matching (beyond age and sex)	Age (diagnosis)	Response rate	Breastfeeding definition	Minimal duration of breastfeeding	Crude OR (95% CI)	Adjusted OR (95% CI)
		Cases	Controls							
Thompson et al, 2000 (29), United Kingdom	3	24	186 population based nested in 2 cohorts	Yes²; by social class	Unknown	Irrelevant (data reviewed from medical records)	No exact definition	No exact definition	0.4 (0.15, 1.03)	NA
Persson et al, 1993 (27), Sweden (Stockholm)	3	152	305 population-based controls	No	Children and adults	Cases, 83%; controls, 78%	No exact definition	2 mo	1 (0.61, 1.63)⁷	NA
Wurzelmann et al, 1994 (30), United States (North Carolina)	3	322	262 neighbors of the same sex, race, and age of patients	Yes²; inherent in control selection	Mostly adults	Cases, 73%; controls, 80%	No exact definition	No exact definition	1 (0.67, 1.5)⁷	NA
Gruber et al, 1996 (25), United States (New York)	3	54	90 controls selected from offspring of friends, neighbors, and acquaintances of mothers of the cases	Yes²; inherent in control selection	Children and young adults (diagnosis under the age of 22 y)	72%	No exact definition	No exact definition	0.6 (0.29, 1.3)	NA
Klein et al, 1998 (5), Israel (Tel-Aviv)	3	33	144 population and hospital outpatient controls	Yes²; by country of origin and residential neighborhood	Children and adults	Unknown	No exact definition	1 mo	0.67 (0.28, 1.6)⁸	NA
Thompson et al, 1995 (28), United Kingdom	3	1396	1396 friends or neighbors of the same sex, ethnic origin, and age	Yes; by ethnicity	Mostly adults	21%	No exact definition	No exact definition	1.04 (0.9, 1.25)	NA

[1] Studies in quality groups 1 and 2 are organized according to year of publication. Studies in quality group 3 are organized according to decreasing quality. OR, odds ratio; NA, not available.

[2] Matching was not treated statistically in analysis.

[3] Negative association trend between duration of breastfeeding and risk of Crohn disease.

[4] The OR and 95% CI were calculated according to the data presented in the article.

[5] For all ORs, ORs and 95% CIs adjusted for the specified variable were calculated by using the fixed-effects model described by Greenland (35) according to the data presented in the article.

[6] Although 604 controls were included in the study, only half of them (302) were included in the matched analysis.

[7] The exact OR was not mentioned. Because the article indicated that no significant difference was found, we assumed the OR to be ≈1. The CI was calculated according to the sample size by assuming an exposure rate of 20% in the control group.

[8] The OR and 95% CI were calculated according to the data presented in the article, assuming that all patients and controls provided information regarding breastfeeding.

TABLE 3
Quality grading of studies[1]

Quality group and study	UC	CD	Recruitment of controls by investigators rather than cases themselves	Breastfeeding information always obtained from mother or older relative	Diagnosis always confirmed by a physician	Response rate was mentioned and was ≥80%
1 (highest quality)						
Acheson and Truelove, 1961 (17)	+	−	+	+	+	+
Bergstrand and Hellers, 1983 (18)	−	+	+	+	+	+
Koletzko et al, 1989 (20)	−	+	+	+	+	+
Ekbom et al, 1990 (24)	+	+	+	+	+	NA
Koletzko et al, 1991 (26)	+	−	+	+	+	+
Rigas et al, 1993 (21)	+	+	+	+	+	+
2 (intermediate quality)						
Whorwell et al, 1979 (22)	+	+	+	+	+	−
Gilat et al, 1987 (4)	+	+	+	+	+	−
Kono et al, 1994 (23)	+	−	+	+	+	−
Urashima et al, 1999 (33)	+	+	+	+	+	−
3 (lowest quality)						
Corrao et al, 1998 (19)	+	+	+	−	+	−
Thompson et al, 2000 (29)	+	+	+	+	−	NA
Persson et al, 1993 (27)	+	+	+	−	+	−
Wurzelmann et al, 1994 (30)	+	+	+	−	+	−
Gruber et al, 1996 (25)	−	+	−	+	+	−
Klein et al, 1998 (5)	+	+	+	−	+	−
Thompson et al, 1995 (28)	+	+	−	−	+	−

[1] See text for grading considerations. Studies in quality groups 1 and 2 are organized according to year of publication. Studies in quality group 3 are organized according to decreasing quality. UC, ulcerative colitis; CD, Crohn disease; NA, not applicable.

by Bergstrand and Hellers (18), we think that these missing data are probably of high importance.

As was mentioned previously in this report, most studies did not define duration and exclusivity of breastfeeding precisely, and it was not clear whether exclusive breastfeeding was being compared with nonexclusive breastfeeding or whether nonexclusive breastfeeding was being compared with exclusive bottle-feeding. Thus, it cannot be stated whether the absence of breastfeeding is the risk factor for IBD or the presence of bottle-feeding. It is also worth noting that this inadequate definition of breastfeeding duration and exclusivity can lead to nondifferential misclassification, which might obscure the protective association between breastfeeding and IBD (39). A good example for this type of nondifferential misclassification can be drawn from the study of Ekbom et al (24). That study, although conducted with almost perfect methodology, defined breastfeeding according to medical records, which merely documented breastfeeding status in the first few days after labor. It is possible that a significant portion of mothers that were assigned in this study as

exclusive breastfeeders moved to partial breastfeeding or totally gave up breastfeeding a few days later. Thus, the lack of association found in that study can actually be an underestimation of an existing association that would have been discovered had breastfeeding status been recorded for a longer duration.

Confounding was not treated statistically in most of the studies. Confounding can potentially bias the results, but the few studies in which adjusted and crude OR were calculated (17, 19–21) showed little difference between the adjusted OR that controls for various confounders (diarrheal disease during infancy, sex, age, race, birthplace, sibship size, birth order, maternal age, smoking, and the use of oral contraceptives) and the crude OR. We, therefore, believe that the lack of adjustment to confounders in most of the studies probably did not lead to a significant bias of the results.

Most of the studies matched case with control subjects for sex and age. Some studies also matched other variables (region or

TABLE 4
Pooled estimates for correlation between breastfeeding and risk of ulcerative colitis (UC) and Crohn disease (CD)[1]

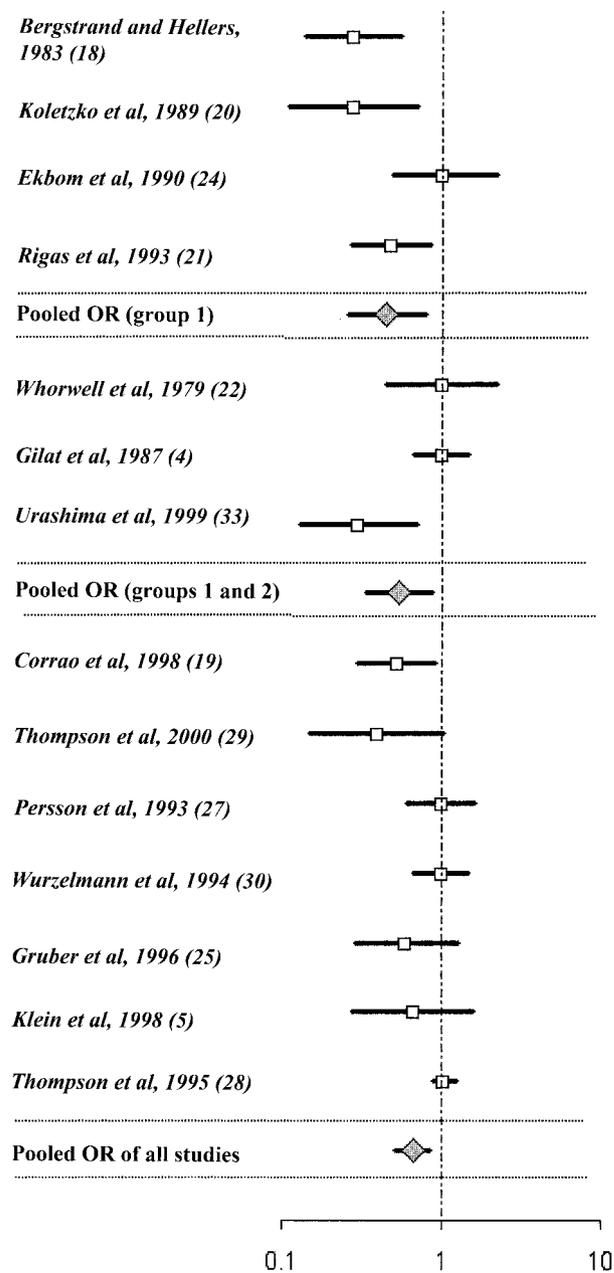|		CD				UC		
		OR (95% CI)				OR (95% CI)		
	n of studies	Fixed-effects model	Random-effects model	*P* for heterogeneity	*n* of studies	Fixed-effects model	Random-effects model	*P* for heterogeneity
Quality group 1	4	0.46 (0.32, 0.65)	0.45 (0.26, 0.79)	0.063	4	0.57 (0.41, 0.79)	0.56 (0.38, 0.81)	0.268
Quality groups 1 and 2	7	0.63 (0.49, 0.80)	0.55 (0.34, 0.87)	0.003	8	0.64 (0.51, 0.80)	0.61 (0.46, 0.83)	0.093
Quality groups 1, 2, and 3	14	0.86 (0.76, 0.96)	0.67 (0.52, 0.86)	<0.001	14	0.83 (0.75, 0.95)	0.77 (0.61, 0.96)	0.004

[1] OR, odds ratio
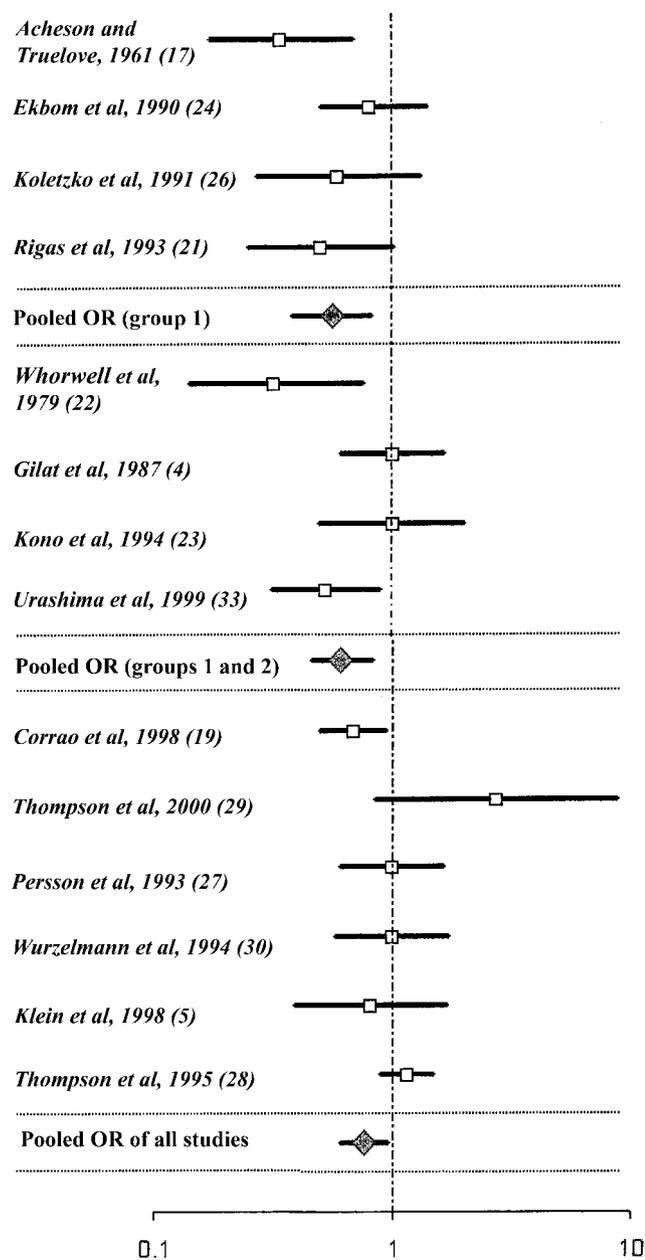
FIGURE 1. Association between breastfeeding and ulcerative colitis. The *x* axis represents the odds ratio (OR) depicted on a logarithmic scale. ORs are represented by small white squares, and 95% CIs are represented by lines. Pooled ORs for group 1, groups 1 and 2, and all studies were calculated by using the random-effects model whenever possible. Otherwise, calculation was performed according to the fixed-effects model. Studies in quality groups 1 (highest) and 2 are organized according to date of publication. Studies in quality group 3 are organized according to decreasing quality.

FIGURE 2. Association between breastfeeding and Crohn disease. The *x* axis represents the odds ratio (OR) depicted on a logarithmic scale. ORs are represented by small white squares, and 95% CIs are represented by lines. Pooled ORs for group 1, groups 1 and 2, and all studies were calculated by using the random-effects model whenever possible. Otherwise, calculation was performed according to the fixed-effects model. Studies in quality groups 1 (highest) and 2 are organized according to date of publication. Studies in quality group 3 are organized according to decreasing quality.

country of birth, residential neighborhood), and in some matching was inherent in the control subject selection (neighbors, acquaintances, siblings). However, only in a few of the studies was matching statistically treated through conditional logistic regression or McNemar test. The lack of this statistical treatment in most studies can lead to bias of the OR toward unity (no relation); thus, the result presented in these studies might lead to underestimation of the protective association between breastfeeding and IBD (40).

Finally, publication bias, which results from a tendency to publish only significant data, constitutes a potential problem in

every meta-analysis. The funnel plots of both CD and UC show that most of the studies have about the same precision. The funnel plot of the CD studies has an asymmetric appearance. This asymmetry is supported by the low *P* value (0.003) result in the test for skewed funnel plot for CD. In meta-analysis of observational studies, however, larger sample sizes do not necessarily indicate a higher validity (36). For example, it can be seen that the distorted shape of the plot is caused primarily by the study of Thompson et al (28), which, as was previously outlined, has the

TABLE 5
Summary estimates of the odds ratios (ORs) for the association of ulcerative colitis (UC) and Crohn disease (CD) with breastfeeding according to study characteristics[1]

		UC			CD	
Study characteristic	n	Pooled OR (95% CI)	P for heterogeneity	n	Pooled OR (95% CI)	P for heterogeneity
Quality grade						
1 (highest)	4	0.56 (0.38, 0.81)	0.26	4	0.45 (0.26, 0.79)	0.06
2	4	0.67 (0.41, 1.1)	0.06	3	0.71 (0.35, 1.44)	0.04
3	6	0.98 (0.75, 1.28)	0.08	7	0.88 (0.72, 1.08)	0.22
Control characteristics						
Hospital based	4	0.6 (0.41, 0.87)	0.15	3	0.71 (0.47, 1.09)	0.11
Population based	6	1.02 (0.81, 1.29)	0.27	8	0.68 (0.48, 0.97)	0.001
Mixed	4	0.64 (0.4, 1.02)	0.09	3	0.59 (0.29, 1.19)	0.12
Adjustment for confounding[2]						
Yes	4[3]	0.59 (0.46, 0.76)	0.55	3	0.48 (0.32, 0.72)	0.32
No	13	0.78 (0.62, 0.99)	0.003	14	0.68 (0.53, 0.88)	<0.001
Matching						
Yes	9	0.92 (0.73, 1.15)	0.45	10	0.64 (0.46, 0.89)	<0.001
No	5	0.57 (0.38, 0.84)	0.05	4	0.7 (0.47, 1.04)	0.14
Breastfeeding definition						
Exclusive	4	0.53 (0.34, 0.82)	0.11	3	0.8 (0.5, 1.3)	0.36
Nonexclusive[4]	1	0.53 (0.31, 0.89)	NA	1	0.46 (0.19, 1.1)	NA
Not defined	10	0.91 (0.73, 1.12)	0.11	11	0.66 (0.5, 0.87)	<0.001
Participants' age						
Children	4	0.66 (0.46, 0.93)	0.26	4	0.48 (0.25, 0.92)	0.005
Adults	4	0.9 (0.68, 1.2)	0.08	4	0.92 (0.7, 1.2)	0.16
Mixed	6	0.75 (0.44, 1.28)	0.008	6	0.62 (0.4, 0.96)	0.06

[1] NA, not applicable.

[2] Three studies were included in both categories.

[3] For the study by Acheson and Truelove (17), OR adjusted for residence region was included in the pooled analysis.

[4] For the study by Urashima et al (33), separate ORs were calculated for exclusive and nonexclusive breastfeeding.

largest sample size but suffers from some important methodologic problems. In addition, most of the studies were performed for both CD and UC; thus, it is unlikely that publication bias exists for one but not for the other. Nevertheless, publication bias cannot be ruled out in this meta-analysis.

In conclusion, our study supports the hypothesis that breastfeeding provides protection against CD and UC development. However, it does not presume to provide an exact estimate of the OR for a certain definition of breastfeeding, but rather to provide a rough measure of the relation between breastfeeding and the risk of IBD. Our thought is that, because of a result of nondifferential misclassification, which, as we stated earlier, is inherent in many of the studies reviewed, the actual effect of breastfeeding is higher than the one estimated here. Furthermore, most of the best quality studies showed a significant protective effect. Nevertheless, because the effect found was minor and inconsistent,
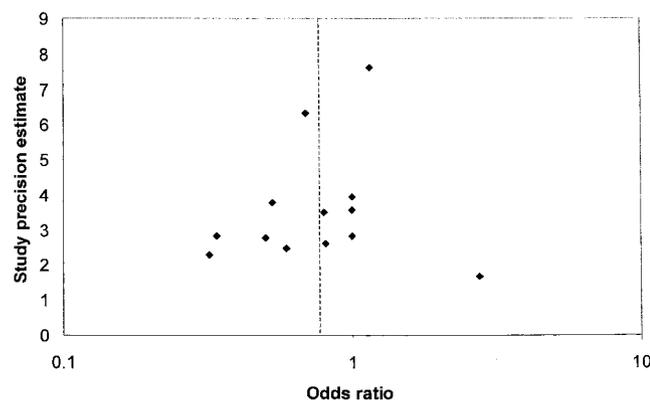

FIGURE 3. Funnel plot of precision estimates [calculated by using the inverse of the SE of ln(odds ratio); ie, the higher the estimate, the more precise the study] from studies that explored the association between breastfeeding and ulcerative colitis against their odds ratio. The vertical dashed line is the summary estimate of the odds ratio for all studies.
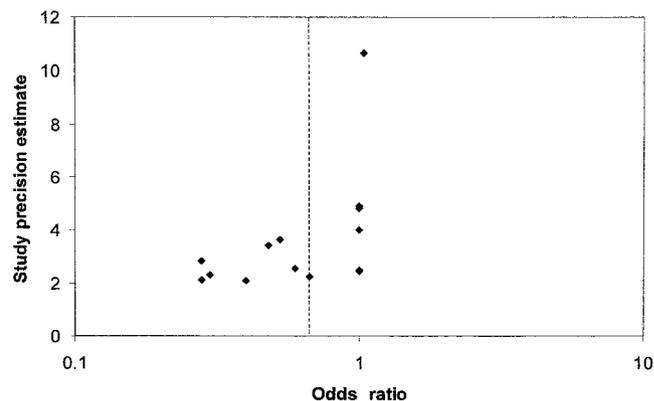

FIGURE 4. Funnel plot of precision estimates [calculated by using the inverse of the SE of ln(odds ratio); ie, the higher the estimate, the more precise the study] from studies that explored the association between breastfeeding and Crohn disease against their odds ratio. The vertical dashed line is the summary estimate of the odds ratio for all studies.

our study should not be regarded as final proof of this hypothesis. We think that a well-performed, documented prospective study should be held. Studies of high-risk populations that will specifically address the influence of breastfeeding (as well as its duration) are of particular importance. Because there is a clear genetic predisposition to IBD, these populations should probably be composed of families that include persons who already have IBD [such as the studies conducted by Koletzko et al (20, 26)]. That kind of study will enable the generation of breastfeeding recommendations to mothers of infants with a history of IBD in first-degree relatives. ☙

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