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Evaluating the efficacy of breastfeeding guidelines on long-term outcomes for allergic disease

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Short Title: Effects of breastfeeding on asthma and allergy

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Abstract

Background: WHO guidelines advocate breastfeeding for six months, and EAACI recommends exclusive breastfeeding for 4-6 months. However, evidence for breastfeeding to prevent asthma and allergic disease is conflicting. We examined whether following recommended breastfeeding guidelines alters the long-term risks of asthma, eczema, rhinitis, or atopy.

Methods: The effect of non-exclusive (0, >0-6, >6 months), and exclusive breastfeeding (0, >0-4, >4 months) on repeated measures of asthma (10, 18 years), eczema, rhinitis, and atopy (1-or-2, 4, 10,18 years) risks were estimated in the IoW cohort (*n*=1456) using log-linear models with generalised estimating equations. The Food Allergy and Intolerance Research (FAIR) cohort (*n*=988), also from the IoW, was examined to replicate results.

Results: Breastfeeding (any or exclusive) had no effect on asthma and allergic disease in the IoW cohort. In the FAIR cohort, any breastfeeding for >0-6 months protected against asthma at 10 years (RR=0.50, 95%CI=0.32-0.79, *p*=0.003) but not other outcomes, while exclusive breastfeeding for >4 months protected against repeated rhinitis (RR=0.36, 95%CI=0.18-0.71, *p*=0.003). Longer breastfeeding was protective against late-onset wheeze in the IoW cohort.

Conclusion: The protective effects of non-exclusive and exclusive breastfeeding against

long-term allergic outcomes were inconsistent between these co-located cohorts, agreeing with previous observations of heterogeneous effects. Although breastfeeding should be recommended for other health benefits, following breastfeeding guidelines did not appear to afford consistent protection against long-term asthma, eczema, rhinitis or atopy. Further research is needed into the long-term effects of breastfeeding on allergic disease.

Introduction

The aetiology of allergy is multifactorial and comprises both genetic and environmental components. It is now recognised that the early life environment, including nutrition, has considerable effect on risk of several diseases including asthma and allergy. Early life nutrition can be provided by breastfeeding or formula feeds, followed by the introduction of complementary foods and weaning. Immunomodulatory breast milk components such as maternal antibodies are absent from formula, and also differ between mothers and between breast milk samples(3).

Breastfeeding for at least six months is recommended, unless contraindicated, by World Health Organisation (WHO) guidelines (4). Exclusive breastfeeding is advised by the European Academy of Allergy and Clinical Immunology (EAACI) for at least four to six months (5), and EAACI guidelines state that “Breastfeeding has many benefits for mother and child and is therefore recommended for all infants. There is a small amount of evidence to support breastfeeding as a means of preventing the development of food allergy”. Although there is evidence that breastfeeding alters risk of several adult diseases, the potential benefits of breastfeeding for allergic disease are controversial. Additionally, the effect of breastfeeding according to the current clinical guidelines on long-term outcomes for allergic disease has not previously been investigated.

Breastfeeding has been studied before in the Isle of Wight (IoW) birth cohort. Exclusive breastfeeding for the first three months of life is protective against early childhood atopic wheeze (6). Breastfeeding for over four months is associated with increased lung volume at age 10 (7). Increased lung function was also seen at age 18 but stratifying by height reduced this association (8). Additionally, prolonged breastfeeding mitigated the impact of maternal smoking during pregnancy and recurrent lower respiratory tract infection (RLRTI) on asthma risk (repeated measures up to age 10) (9). In the FAIR cohort, breastfeeding type (exclusive or non-exclusive) or duration has no effect on food allergy prevention in early childhood (10). However the long-term effect of breastfeeding on repeated asthma, eczema, rhinitis, and atopy up to age 18 is uncertain.

In general, studies that report a protective effect of breastfeeding on allergic disease look at allergic outcomes in early childhood, and studies of the long-term effects are scarce, therefore the role of breastfeeding in allergic disease throughout adolescence needs further investigation (11). In particular, asthma diagnosis in early childhood is primarily based on wheeze so studies reporting an early protective effect of breastfeeding on 'asthma' may not identify those who continue to exhibit persistent asthma symptoms in adulthood.

Crucially, categorisation of breastfeeding duration is widely variable, complicating inter-study comparisons. Evidence for the effects of breastfeeding is inconsistent and often limited by the absence of a non-breastfed reference category (12, 13). This study aims to evaluate how appropriate breastfeeding guidelines are specifically for long-term asthma and allergic disease risk, in a longitudinal prospective birth cohort and validation cohort. We hypothesised that guideline breastfeeding would be protective against development of asthma and allergic disease.

Materials and Methods

The Isle of Wight (IoW) cohort

The IoW birth cohort (*n*=1456) was set up in 1989 to prospectively investigate the development of asthma and allergic disease. Mothers were approached at birth and following exclusion of subjects due to adoption, follow-up refusal, or perinatal deaths 95% of children were enrolled. In line with ethical approval, written parental consent was obtained for all participants and follow-ups were conducted at 1, 2, 4, 10, and 18 years. The cohort has been described in detail elsewhere (14). All subjects with outcome data for at least one follow-up were included in analyses (*n*=1496, 1496, 1495, and 1289 for asthma, eczema, rhinitis, and atopy respectively). Since the 1-year and 2-year follow-ups were completed in a relatively small time window, they were combined (reported as 1-or-2 years).

Variable definitions

Breastfeeding duration data were obtained retrospectively at the 1-and-2 year follow-ups. The minimum six months breastfeeding recommended by the WHO guidelines was used to categorise breastfeeding into 'no breastfeeding', '>0-6 months', and '>6 months' breastfeeding.

Duration of exclusive breastfeeding duration was defined as the age at which formula feeding or solid foods were first introduced. According to EAACI guidelines (5), mothers should attempt to breastfeed exclusively from birth until four months unless contraindicated. Exclusive breastfeeding was therefore categorised into 'no exclusive breastfeeding, '>0-4 months', and '>4 months'.

Socioeconomic status was determined by combining parental occupation at birth, number of children in the index child's bedroom, and family income as described previously (7). Maternal smoking during pregnancy was recorded at birth. RLRTI was defined as two or more episodes of parental report of chest infections, based on productive cough lasting for five or more days in the preceding 12 months. Antibiotic usage and wheezing were not prerequisites for the diagnosis of RLRTI. Information on RLRTI was collected at 1 and 2 years. Other variables included sex, birth order, season of birth, birthweight, gestational age, mode of delivery and parental history of allergy (ever).

Asthma definition required both "history of physician-diagnosed asthma" and either "wheezing or whistling in the chest in the last 12 months" or "asthma treatment in the previous 12 months". As asthma is difficult to diagnose with confidence in early childhood, we only used 10 and 18-year data for asthma. Eczema was defined as chronic or chronically relapsing itchy dermatitis lasting >6 weeks with characteristic morphology and distribution, following Hanifin and Rajka criteria (16). Rhinitis was defined as a problem with sneezing, or a runny or blocked nose without cold or flu in the last year (17). Atopy was determined by skin prick testing as described previously (17).

The Food Allergy and Intolerance Research (FAIR) cohort was used for validation. The FAIR cohort (*n*=988) was established in 2001, and at present has follow-up to 10 years of age. The same definitions and methods were used in the FAIR cohort, except that maternal education was used as a proxy for maternal socioeconomic status, and no data were available for parental atopy or RLRTI. The FAIR cohort has been described in detail elsewhere (18). IoW results were validated in FAIR participants at ages 1, 2, 3, and 10 years. Loss to follow-up was minimal for both cohorts (Figure 1).

Statistical analyses

The generalised additive models (GAM) implemented in our analyses utilised splines to semi-parametrically describe the association of asthma risk with breastfeeding duration in the IoW cohort. The PROC GAM procedure in SAS (2) was used to fit the model. This procedure also provided a test on the statistical significance with respect to a linear association of asthma risk with breastfeeding duration.

χ^2 tests were used to test the independence of factors associated with breastfeeding duration (maternal socioeconomic status, maternal smoking during pregnancy, sibling order, season of birth, sex, parental history of allergy, RLRTI, birthweight, gestational age, and mode of delivery (9, 19-24)), to identify potential confounding factors. Where available these factors were also tested in the FAIR cohort to assess whether any associations have changed over time on the Isle of Wight. Adjusted models therefore controlled for sex, birth order, maternal socioeconomic status, maternal smoking, season of birth, parental history of allergic outcome (asthma, eczema, rhinitis, or atopy) and in the case of asthma also RLRTI. Relative risks (RRs) of breastfeeding duration on repeated asthma, eczema, rhinitis, and atopy – both adjusted and unadjusted models – were calculated using log-linear models via generalised estimating equations.

For asthma only long-term outcomes were investigated, following previous analyses of breastfeeding on early wheeze in the cohort (6). In the IoW cohort asthma at 10 and 18 years and in the FAIR cohort asthma at age 10 were examined. Log linear models were used to calculate RRs for the 10-year data in the FAIR cohort. All statistical analyses used SPSS software (version 22.0.0, IBM).

Wheeze trajectories

To analyse the effect of breastfeeding duration on subtypes of early wheeze, wheeze trajectories were constructed based on presence/absence of wheeze ages 1-or-2 and 4 in the IoW cohort (25). These categories were never wheezed, early transient wheeze, late wheeze, and persistent wheeze (see Table S1). Log linear models were used to examine the effect of non-exclusive and exclusive breastfeeding on the trajectories, with 'never wheezed' as the reference trajectory. Most adjusted models reached singularity for both non-exclusive and exclusive breastfeeding, so adjusted models only control for sex and RLRTI.

Results

Cohort characteristics

Characteristics of the IoW and FAIR cohorts are presented in Table 1. In the IoW cohort there were approximately equal numbers of male and female participants. The majority of mothers initially tried to breastfeed, were non-smokers, and did not suffer from asthma, eczema, rhinitis, or atopy (Table 1), and 21% of mothers did not breastfeed at all. The prevalence of asthma, eczema, rhinitis, and atopy at 18 were 17.7%, 12.3%, 35.8%, and 41.4% respectively. The FAIR cohort included more mothers who did not breastfeed (31.3%), and prevalence of asthma, eczema, rhinitis, and atopy at 10 were 18.7%, 31.2%, 23.9%, 24.6% respectively. For asthma at age 10, GAM indicated a potential non-linear relationship (p=0.0613, Figure S1), which implies assuming linearity between breastfeeding duration and asthma risk is not appropriate. Furthermore, the non-linear pattern suggested a categorisation of breastfeeding around the 30 weeks time point, which agrees with the 6 months breastfeeding recommendation by the WHO.

Confounding factors

Potential confounders were tested for association with breastfeeding duration (Tables S2 and S3). In both cohorts, socioeconomic status, maternal smoking during pregnancy, sibling order, and (in the IoW cohort) RLRTI were associated with breastfeeding duration. These were therefore controlled for in the adjusted models, alongside well-evidenced confounders from the literature.

Primary Analysis: Non-exclusive breastfeeding

The effect of non-exclusive breastfeeding on allergic outcomes was examined using both adjusted and unadjusted models. These analyses showed no significant effect of non-exclusive breastfeeding duration across the ages of 1-or-2, 4, 10, and 18 on asthma, eczema, rhinitis or atopy in the IoW cohort (Table 2).

The validation cohort replicated the non-significant results seen in the IoW cohort for eczema, rhinitis, and atopy. However, FAIR children who were breastfed for >0-6 months were only half as likely to have asthma at age 10 as those not breastfed (Table 2). This effect was seen in both unadjusted and adjusted (RR=0.50, 95%CI 0.32-0.79, p=0.003) models and remains significant after Bonferroni correction (four allergic outcomes tested).

Secondary analysis: Exclusive breastfeeding

Relative to participants never exclusively breastfed, participants exclusively breastfed for less than or greater than 4 months did not differ in their risks of long-term asthma or allergic disease (Table 3). In unadjusted models, risk of eczema was higher among children exclusively breastfed for >4 months relative to non-exclusive breastfeeding in the IoW cohort (RR=1.44, 95%CI 1.06-1.95, p=0.018). This did not remain significant after adjustment or after Bonferroni correction (four allergic outcomes tested).

In the validation cohort, exclusive breastfeeding for >4 months was protective against repeated measures of rhinitis relative to non-exclusive breastfeeding, in both unadjusted and adjusted (RR=0.36, 95%CI 0.18-0.71, *p*=0.003) models (Table 3). Additionally, exclusive breastfeeding for >0-4 months was protective against asthma at 10 years in the adjusted model (RR=0.68, 95%CI 0.46-0.99, *p*=0.045). This remained significant for rhinitis but not asthma after Bonferroni correction (four allergic outcomes tested).

Analysis at each time point for non-exclusive and exclusive breastfeeding

Analysis of each outcome at each time point was done to determine whether inter-cohort differences were due to differences in length of follow-up. Effects of non-exclusive and exclusive breastfeeding remained different between the two cohorts even at similar follow-up ages (Table S4).

Early wheeze Trajectories

In the IoW cohort adjusted models, non-exclusive breastfeeding (Table S5) for >6 months was protective against falling into the late wheeze trajectory, relative to never wheezing (RR=0.54, 95%CI 0.32-0.90, *p*=0.019). Similarly, exclusive breastfeeding (Table S6) for >4 months was borderline significantly protective against late wheeze (RR=0.57, 95%CI 0.32-1.01, *p*=0.052).

In the FAIR cohort there were no significant effects of breastfeeding on wheeze trajectories in adjusted analyses (Table S5 and S6). However, >6 months non-exclusive breastfeeding (Table S5) had the same direction of effect (protective) against late-onset wheeze, as observed in the IoW cohort, but did not reach statistical significance.

Discussion

This study found inconsistent protective effects of breastfeeding duration on long-term allergic outcomes. Non-exclusive breastfeeding duration had no significant effect on long-term allergic disease in the IoW cohort, though in the validation (FAIR) cohort >0-6 months breastfeeding was protective against asthma until the age of 10. Exclusive breastfeeding duration did not affect long-term allergy in the IoW birth cohort, though >4 months exclusive breastfeeding protected against rhinitis in the validation cohort. Non-exclusive breastfeeding for >6 months was significantly protective against the late onset early wheeze trajectory in the IoW cohort, and had the same direction of effect in the validation cohort. This divergence in long-term effects on allergic disease, even between two cohorts from the Isle of Wight, shows that current breastfeeding duration guidelines are not consistently protective, and highlights the need for further research.

Primary analysis: Non-exclusive breastfeeding

Non-exclusive breastfeeding duration did not significantly affect development of allergic disease in later childhood and early adulthood in the IoW cohort, suggesting the previously observed protective effects against early wheeze (6) do not persist. WHO guidance advocates breastfeeding for ≥6 months, and whilst this may provide numerous other health benefits, there is no evidence for the IoW cohort that breastfeeding duration affects longitudinal risk of asthma or allergic disease.

These results were largely reproduced in the validation cohort, although in FAIR a protective effect of non-exclusive breastfeeding for >0-6 months was observed, relative to no breastfeeding, against asthma (age 10). This effect is not significant for >6 months which does not support a dose response effect, although numbers were small in this category. Participation at the follow-up time points was high for both cohorts (Figure 1)

so it seems unlikely that the inconsistent results between the two cohorts are due to loss to follow up. However the analysis of asthma at age 10 in the FAIR cohort only included 460 individuals, which is significantly fewer than in the analyses for the other outcomes so the possibility of selection bias remains.

Previous comparisons report differences in prevalence of wheeze, rhinitis, and allergic sensitisation between the IoW and FAIR cohorts (18), which were established on the same island 12 years apart, suggesting the inconsistent effects on asthma may reflect general changes in allergic disease prevalence over time. Additionally, breastfeeding practices are different between the two cohorts and could contribute to the inconsistencies reported here. Whilst the definition of non-exclusive breastfeeding is the same for both cohorts, other differences exist. Data on RLRTI and parental atopy were unavailable for FAIR, and FAIR participants have not reached 18 years – any protective effect of breastfeeding against wheeze (6, 26) may persist to 10 but not 18 years. Our data on long-term allergic outcomes span puberty. It could be possible that analysing both sexes together has masked different effects of breastfeeding duration on asthma in each sex, since puberty is known to have different effects on asthma in males and females (27). However, in the IoW cohort, stratifying the 18-year asthma analyses by sex revealed no significant effect of breastfeeding duration on asthma in either males or females (data not shown). Combining the two cohorts was considered for meta-analysis and would increase the statistical power of analyses, however this was not suitable due to the differences between the two cohorts.

Our findings agree with several previous studies. While some studies have shown a significant protective effect, the majority of these are limited by study design (28), no non-breastfed reference category (12, 13, 29, 30), small study size (29, 31), or short

follow-up duration (12, 26, 32, 33). A cluster-randomised trial promoting prolonged and exclusive breastfeeding found that 6.5-year-old children in the interventional regions had increased prevalence of prolonged breastfeeding without a significant reduction in allergic symptoms (34), in agreement with our findings. The prospective study design used in our study is advantageous as it limits information bias. Additionally, our analysis of outcomes using repeated measures allows persistence of outcomes to be taken into account. Some studies report protective effects of breastfeeding against allergy at younger ages, though we report that protective effects do not persist to adulthood. Breastfeeding protects against early childhood wheeze (26, 35), but not all wheezing children go on to develop asthma (36). This protective effect on early wheeze has been observed in the IoW cohort previously (6). Studies reporting protective effects against childhood asthma may therefore observe a protective effect against wheeze, not asthma, which is likely to be virus-induced and indicative of the immunological protection that breastfeeding confers. Alternatively, breastfeeding could protect against asthma through reduction in RLRTI, though removing RLRTI infections from our model did not materially alter results (Table S7), and in the IoW cohort the protective effect of longer duration breastfeeding against the late-onset wheeze trajectory remained significant while adjusting for RLRTI, suggesting that the potential effect of breastfeeding on wheeze prevention is not due to protection from RLRTIs.

Additionally the analysis of early wheeze trajectories in the IoW cohort suggests a protective effect of longer non-exclusive duration breastfeeding against late wheeze (children who do not wheeze at 1-or-2 but go on to wheeze at 4 years of age) but not persistent wheeze (children who wheeze at 1-or-2 years and 4 years) relative to never wheeze. The same direction of effect is seen in the validation cohort although this does not reach statistical significance. This suggests that breastfeeding may be protective

against certain subtypes of asthma, in particular the late-onset wheeze trajectory, which has been associated with increased allergic sensitization (37) and therefore may represent subjects likely to subsequently develop asthma. This analysis could explain the inconsistent results seen in this study, and also in the literature. Future research should examine breastfeeding relative to early wheeze trajectories.

Categorisation of breastfeeding duration in previous studies varies widely, and any categorisation has limitations. Firstly, some studies suggest a non-linear relationship between breastfeeding duration and allergic disease (32, 38). Broad categorisation using guideline recommendations may therefore mask non-linear relationships. Furthermore, women who breastfed briefly may say that they did not breastfeed at all, or conversely feel guilty and exaggerate their answer to one week. To address this issue, analyses were rerun with a separate '1 week' breastfeeding category: results for all outcomes were similar to the primary analysis (data not shown), suggesting that potential bias due to misclassification of women between 0-1 weeks is unlikely to affect the results of our analysis.

Another potential limitation is that we were unable to examine reverse causality: a mother's decision to breastfeed may be influenced by early symptoms of allergy in the infant. This could mask any potential effects of breastfeeding on allergy, or result in an apparent increase in risk with prolonged breastfeeding. Few studies examine causality, though one study found early signs of atopy increases duration of exclusive breastfeeding (39). IoW breastfeeding practices recorded at 1 and 2 years after birth could be subject to a degree of recall bias, however recall of breastfeeding duration has been shown to be quite accurate even 20 years after delivery (40). Non-exclusive

breastfeeding does not consider concurrent feeding methods, so a secondary analysis was performed examining exclusive breastfeeding.

Secondary Analysis: Exclusive breastfeeding

Exclusive breastfeeding according to EAACI guidelines provided long-term protection against allergy, though this was inconsistent between cohorts. In the IoW cohort, exclusive breastfeeding duration had no significant effect on long-term allergy, however in the FAIR cohort, exclusive breastfeeding for >4 months was strongly protective against rhinitis, and exclusive breastfeeding for >0-4 months was nominally protective against asthma. For eczema, and atopy our results agree with current research, which suggests that delayed introduction of solids has no benefit; whilst for rhinitis prolonged exclusive breastfeeding may be protective. The significant protective effect against rhinitis was seen only in the validation cohort and is difficult to interpret, as this cohort's rhinitis definition does not differentiate between atopic and non-atopic symptoms. With the publication of the LEAP study(46) and the Consensus statement on peanut introduction(47) changes in the timing of introduction of solid foods is recommended, particularly the introduction of solid foods and peanuts before 6 months of age. This paper is reassuring in the fact that 6 months exclusive breasting does not seem to be important for allergy prevention.

Non-exclusive breastfeeding had a stronger protective effect than exclusive breastfeeding against asthma at 10 years in the FAIR cohort (RR=0.50 vs. RR=0.68). Non-exclusive breastfeeding includes infants who are breastfeeding whilst introducing formula feeds, solid food or cow's milk so perhaps this could explain the inconsistency in results between the two cohorts. Solid foods contain many compounds which are foreign to infants prior to complementary feeding. Breastfeeding whilst introducing gluten-

containing foods has been shown to reduce the risk of coeliac disease(41). It is unknown whether breastfeeding also protects against immunological intolerance to allergic diseases. In the IoW cohort (Table S8), breastfeeding whilst introducing solid foods reduced the risk of asthma, compared with solid introduction without concurrent breast milk (i.e. ceasing breastfeeding and using alternatives such as formula feeds or cow's milk before introducing solid foods). Additionally concurrent breastfeeding reduced the risk of rhinitis in the FAIR cohort. Again, these results are limited by their inconsistency between the two cohorts – future studies should explore the potentially protective effect of breastfeeding concurrent with food introduction.

Although breastfeeding duration according to guidelines does not consistently protect against long-term allergy, breastfeeding may prevent development of allergy through interactions that were not assessed here. Firstly, breast milk composition is widely variable between mothers (42). Differing concentrations of the main immunoglobulin in human breast milk, Immunoglobulin A (IgA), are associated with altered risk of atopy and allergy (43). Along with other immunomodulatory factors in breast milk, IgA is thought to control binding of antigens to the infant intestinal mucosa, and therefore affects not only systemic bacterial invasion but also early microbial colonisation, altering the infant microbiome (44). Secondly, the role of the breast milk microbiome may be important (45). Thirdly, mode of breastfeeding was not examined in this study: breast milk can be given to infants directly from the breast or indirectly using a pump and bottle. The sucking mechanism differs, and may affect infant lung development and possibly lung function in later life. Few studies examine this mechanism, although a study by Soto-Ramírez *et al.* (26) found that direct breastfeeding resulted in a lower risk of wheeze or cough in the first 12 months compared with bottled breast milk or formula feeds. Finally, very few studies examine the effects of guideline recommendations for breastfeeding on

allergy. Since there is conflicting evidence for the effect of breastfeeding duration on long-term allergic disease, more research examining these guidelines is required.

Conclusion

Breastfeeding duration (exclusive or not) had some protective effects against long term allergic disease, although these varied even between the two co-located cohorts. Breastfeeding duration did not significantly affect risk of allergic disease in the IoW cohort, however in the FAIR cohort non-exclusive breastfeeding for >0-6 months was protective against asthma at 10 years, and exclusive breastfeeding for >4 months protected against repeated rhinitis. Analysis of early wheeze trajectories suggests longer duration breastfeeding is protective against late-onset wheeze. WHO guidelines advocate breastfeeding for ≥6 months, though our results do not suggest this affords protection against long-term asthma, eczema, rhinitis, or atopy. Exclusive breastfeeding for ≥4 months is recommended by EAACI guidelines as protective against allergy disease, and this study found protective effects of exclusive breastfeeding for >4 months against long-term rhinitis in the FAIR cohort. Future studies should examine whether aspects of breastfeeding other than duration are important in preventing long-term asthma and allergic disease.

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Declaration of conflicting interests

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

Authors contributions

VB and GAL contributed to design, performed data analysis and wrote the manuscript. HZ and WK contributed to design, analysis and interpretation of the data. NSR and CV contributed to data analysis and revising the manuscript. JWH contributed to the concept, design, interpretation of the data and review for intellectual content. SHA contributed to the concept, acquisition of data and review for intellectual content. All authors approved the final manuscript.

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Tables and Figures

TABLE 1: Baseline characteristics of participants

Variable	IoW cohort (n=1536)	FAIR alternative variable	FAIR cohort (n= 988)
Non-exclusive breastfeeding duration			
No breastfeeding	283 (21.0%)		276 (31.3%)
>0 to 6 months	711 (52.9%)		398 (45.2%)
>6 months	351 (26.1%)		207 (23.5%)
Exclusive breastfeeding duration			
No breastfeeding	425 (31.2%)		329 (40.5%)
>0 to 4 months	744 (54.6%)		437 (53.8%)
>4 months	193 (14.2%)		46 (5.7%)
Maternal socioeconomic status			
1 (lowest)	209 (15.4%)	Did not finish school	12 (1.2%)
2	240 (17.7%)	School	363 (37.7%)
3	403 (29.7%)	Further education	437 (45.3%)
4	394 (29.0%)	Higher education	152 (15.8%)
5 (highest)	111 (8.2%)		
Maternal smoking during pregnancy			
Yes	384 (25.2%)		211 (22.5%)
No	1137 (74.8%)		726 (77.5%)
Season of birth			
Winter	499 (32.5%)		221 (22.8%)
Spring	364 (23.7%)		256 (26.4%)
Summer	353 (23.0%)		248 (25.6%)
Autumn	320 (20.8%)		244 (25.2%)
Sibling order			
1	510 (42.1%)		401 (41.4%)
2	419 (34.6%)		316 (32.6%)
≥3	283 (23.3%)		252 (26.0%)
Sex			
Male	786 (51.2%)		495 (51.7%)
Female	750 (48.8%)		462 (48.3%)
Parental asthma			
None	1208 (80.4%)		615 (65.1%)
Maternal	147 (9.8%)		168 (17.8%)
Paternal	132 (8.8%)		117 (12.4%)

Both	16 (1.1%)		45 (4.8%)
Parental eczema			
None	1242 (82.7%)		629 (67.1%)
Maternal	164 (10.9%)		193 (20.6%)
Paternal	81 (5.4%)		69 (7.4%)
Both	15 (1%)		47 (5%)
Parental rhinitis			
None	1030 (68.4%)		473 (51.5%)
Maternal	255 (16.9%)		194 (21.1%)
Paternal	176 (11.7%)		164 (17.9%)
Both	44 (2.9%)		87 (9.5%)
Parental atopy		n/a	
None	744 (49.4%)		-
Maternal	377 (25.0%)		-
Paternal	244 (16.2%)		-
Both	142 (9.4%)		-
RLRTI	211 (15.4%)	n/a	-
OUTCOMES			
Asthma at 10	201 (14.7%)	**Asthma at 10**	101 (19.7%)
Asthma at 18	231 (17.7%)		
Eczema at 1 or 2	196 (14.2%)	**Eczema at 1**	168 (18.7%)
Eczema at 4	145 (11.9%)	**Eczema at 2**	204 (23.9%)
Eczema at 10	186 (13.7%)	**Eczema at 3**	163 (18.4%)
Eczema at 18	161 (12.3%)	**Eczema at 10**	258 (31.2%)
Rhinitis at 1 or 2	217 (15.8%)	**Rhinitis at 1**	184 (20.4%)
Rhinitis at 4	65 (5.4%)	**Rhinitis at 2**	141 (16.4%)
Rhinitis at 10	308 (22.6%)	**Rhinitis at 3**	133 (14.9%)
Rhinitis at 18	468 (35.8%)	**Rhinitis at 10**	198 (23.9%)
Atopy at 1 or 2	41 (7.8%)	**Atopy at 1**	20 (2.6%)
Atopy at 4	192 (19.6%)	**Atopy at 2**	54 (8.2%)
Atopy at 10	279 (26.9%)	**Atopy at 3**	76 (11.8%)
Atopy at 18	353 (41.4%)	**Atopy at 10**	145 (24.6%)

Numbers of subjects in each subgroup are shown (with % of total); The full IoW cohort has *n* = 1,536 subjects recruited at birth and *n* = 1,456 available for follow-up; the full FAIR cohort has *n* = 988 subjects recruited at birth; where numbers total to less than this the difference is due to missing data. Column 3 displays variables for the FAIR cohort if different from the IoW cohort.

TABLE 2: Effect of duration of non-exclusive breastfeeding on risk of allergic disease in the IoW and FAIR cohorts

	Duration of non-exclusive breastfeeding (months) (RR; 95 CI; *p* value)		
	0	**>0-6**	**>6**
IoW Unadjusted			
Asthma	Ref.	1.06; (0.80-1.40); 0.709	0.91; (0.65-1.27); 0.563
Eczema	Ref.	1.30; (0.98-1.71); 0.065	1.20; (0.88-1.63); 0.253
Rhinitis	Ref.	0.99; (0.85-1.15); 0.848	0.92; (0.77-1.09); 0.327
Atopy	Ref.	1.03; (0.84-1.26); 1.029	1.05; (0.84-1.31); 0.695
IoW Adjusted			
Asthma	Ref.	1.02; (0.75-1.39); 0.908	0.95; (0.66-1.36); 0.769
Eczema	Ref.	1.32; (0.97-1.80); 0.076	1.10; (0.77-1.57); 0.585
Rhinitis	Ref.	1.01; (0.85-1.20); 0.945	0.90; (0.74-1.10); 0.311
Atopy	Ref.	0.98; (0.80-1.21); 0.869	1.04; (0.83-1.30); 0.733
FAIR Unadjusted			
Asthma	Ref.	**0.57; (0.37 - 0.87); 0.010**	0.82; (0.52 - 1.29); 0.381
Eczema	Ref.	1.05; (0.85-1.30); 0.650	1.09; (0.85-1.40); 0.478
Rhinitis	Ref.	0.93; (0.78-1.11); 0.408	0.86; (0.69-1.07); 0.172
Atopy	Ref.	1.09; (0.78-1.54); 0.605	1.40; (0.97-2.03); 0.069
FAIR Adjusted			
Asthma	Ref.	**0.50; (0.32 - 0.79); 0.003**	0.89; (0.56 - 1.41); 0.614
Eczema	Ref.	1.01; (0.81-1.26); 0.940	1.05; (0.81-1.36); 0.697
Rhinitis	Ref.	1.01; (0.84-1.22); 0.925	0.95; (0.75-1.20); 0.675
Atopy	Ref.	0.97; (0.69-1.35); 0.848	1.14; (0.78-1.67); 0.490

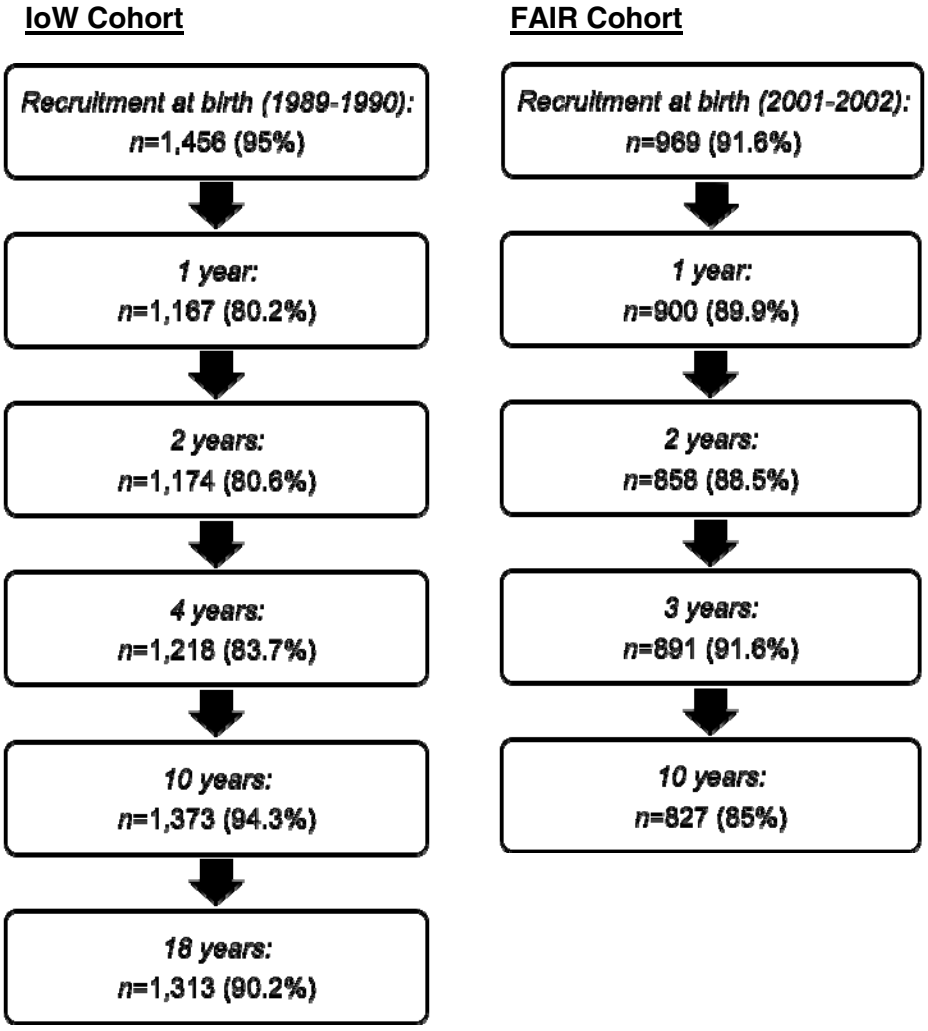
Effect of breastfeeding duration was assessed on asthma (*n*=1087) at ages 10 and 18, and on eczema (*n*=1106), rhinitis (*n*=1109), and atopy (*n*=1052) at ages 1-or-2, 4, 10 and 18 in the IoW cohort; and on asthma (*n*=460) at 10 years, and on eczema (*n*=834), rhinitis(*n*=819), and atopy (*n*=791) at 1, 2, 3 and 10 years in the FAIR cohort. CI: confidence interval. IoW adjusted models controlled for maternal socioeconomic status, maternal smoking during pregnancy, season of birth, sibling order, sex, parental history of relevant allergic disease, and for asthma also RLRTI. Significant effects are shown in bold font. FAIR cohort adjusted models controlled for maternal education, maternal smoking during pregnancy, season of birth, sibling order, sex, and parental history of relevant allergic disease. Significant effects are shown in bold font.

TABLE 3: Effect of exclusive breastfeeding duration on risk of allergic disease in the IoW and FAIR cohorts

	Duration of exclusive breastfeeding (months) (RR; 95 CI; *p* value)		
	0	**>0-4**	**>4**
IoW Unadjusted			
Asthma	Ref.	1.02; (0.78-1.34); 0.873	0.91; (0.62-1.35); 0.648
Eczema	Ref.	1.01; (0.79-1.31); 0.923	**1.44; (1.06-1.95); 0.018**
Rhinitis	Ref.	0.99; (0.85-1.15); 0.892	0.98; (0.81-1.20); 0.867
Atopy	Ref.	1.02; (0.84-1.24); 0.863	1.19; (0.94-1.50); 0.157
IoW Adjusted			
Asthma	Ref.	1.04; (0.76-1.41); 0.812	1.00; (0.66-1.50); 0.982
Eczema	Ref.	1.11; (0.83-1.48); 0.495	1.37; (0.97-1.93); 0.075
Rhinitis	Ref.	1.04; (0.88-1.23); 0.670	1.00; (0.81-1.24); 0.978
Atopy	Ref.	0.94; (0.77-1.15); 0.533	1.09; (0.86-1.37); 0.487
FAIR Unadjusted			
Asthma	Ref.	0.74; (0.51 - 1.08); 0.117	0.21; (0.03 - 1.41); 0.107
Eczema	Ref.	1.05; (0.87-1.28); 0.592	1.10; (0.72-1.66): 0.665
Rhinitis	Ref.	0.96; (0.82-1.14); 0.660	**0.31; (0.15-0.62); <0.001**
Atopy	Ref.	1.19; (0.87-1.62); 0.285	1.11; (0.61-2.04); 0.732
FAIR Adjusted			
Asthma	Ref.	**0.68; (0.46 - 0.99); 0.045**	0.20; (0.03 - 1.37); 0.101
Eczema	Ref.	1.03; (0.84-1.27); 0.746	1.07; (0.70-1.63); 0.762
Rhinitis	Ref.	1.05; (0.88-1.24); 0.614	**0.36; (0.18-0.71); 0.003**
Atopy	Ref.	1.09; (0.80-1.49); 0.574	0.96; (0.52-1.77); 0.884

Effect of exclusive breastfeeding duration was analysed on asthma(*n*=1011) at 10 and 18, and on eczema(*n*=1031), rhinitis (*n*=1034), and atopy (*n*=978) at 1-or-2, 4, 10 and 18 in the IoW cohort; and on asthma at 10, and eczema, rhinitis, and atopy at 1, 2, 3, and 10 years in the FAIR cohort. CI: confidence interval. IoW cohort adjusted models controlled maternal socioeconomic status, maternal smoking during pregnancy, season of birth, sibling order, sex, parental history of relevant allergic disease, and for asthma also RLRTI. FAIR cohort adjusted models controlled for maternal education, maternal smoking during pregnancy, season of birth, sibling order, sex, and parental history of relevant allergic disease. Significant effects are shown in bold font.

Figure 1: IoW and FAIR Cohort participation rates and follow up



Flow diagram demonstrating the IoW and FAIR cohort participation rates at each time-point from birth up to 18 and 10 years respectively. Percentages are calculated out of the number of children born on the IoW during the recruitment period.