BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-025058
Article Type:	Research
Date Submitted by the Author:	27-Jun-2018
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Keywords:	Breastfeeding, Behaviour Problems, Cohort Study

Breastfeeding and behavioural problems: Propensity score matching with a national cohort of infants in Chile

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Short title: Breastfeeding and behaviour

Abbreviations: Encuesta Longitudinal de la Primera Infancia cohort, ELPI; Propensity Score Matching PSM; Child Behaviour Checklist CBCL; World Health Organization WHO; Randomized Control Trial RCT; Attention to Treat ATT

Author Contributions:
Dr. Girard and Prof. Farkas conceptualized the study, carried out the analyses, interpreted the data, drafted the initial manuscript, critically reviewed and revised the manuscript, and approved the final manuscript as submitted. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Data Access, Responsibility, and Analysis: Dr. Girard and Prof. Farkas had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of Interest Statement: The authors have no conflicts of interest relevant to this article to disclose.

Financial Disclosure Statement: The authors have no financial relationships relevant to this article to disclose.

Word Count: 3000

Key words: Breastfeeding; Behaviour Problems; Propensity Score Matching; Epidemiology; Developmental Cohort

Abstract

Importance: Potential effects of breastfeeding on children's behaviour remains an elusive debate given inherent methodological challenges. Propensity score matching affords benefits by ensuring greater equivalence on observable social and health determinants, helping to reduce bias between groups.

Objectives: We examined whether duration of breastfeeding had an impact on children's externalising and internalising behaviours.

Study Design: A cohort study (Encuesta Longitudinal de la Primera Infancia cohort) that included 3,037 Chilean families who were enrolled in 2010. Follow-up data was collected in 2012.

Setting: General community.

Participants: Population-based sample. Eligibility criteria: children born full-term with complete data on matching variables. Matching variables included: healthcare system as a proxy of income, presence of a partner/spouse in the household, maternal age, educational level, IQ, working status, type of work, diagnosis of prenatal depression by a healthcare professional, smoking during pregnancy, delivery type, child sex, weight at birth, incubation following delivery, and child age.

Exposure: Duration of breastfeeding.

Main Outcomes and Measures: Externalising and internalising problems assessed using the Child Behaviour Checklist.

Results: Matched results revealed benefits of any breastfeeding, up to six months, on emotional reactivity and somatic complaints, (mean difference of -1.05, 95% CI, -0.21 to -1.98 and -1.26, 95% CI, -0.52 to -2.00, respectively). Children breastfed between 7-12 months also had reduced scores on emotional reactivity and somatic complaints, in addition to attention problems (mean difference of -1.05, 95% CI, -0.21 to -1.98; -0.83, 95% CI, -0.11 to -1.55; and -0.52, 95% CI, -0.06 to -0.96, respectively). No benefits were observed for children breastfed 13 months or more.

Conclusion: Reduced internalising difficulties and inattention were found in children breastfed up to a year, suggesting that breastfeeding may have beneficial impacts on these areas of development. The magnitude of effect was modest. Extended durations of breastfeeding did not appear to offer any benefits.

Article Summary:

Strengths:

- Use of a quasi-experimental statistical approach to match children with the propensity to be breastfed to those who were not on observable health and social determinates.
- Use of a large Chilean cohort where confounding structure differs from developed countries.
- The inclusion of 14 matching variables including maternal IQ, which is almost double the average amount of variables included in similar studies.

Limitations:

- No specific information was collected on full breastfeeding in this cohort restricting the study to examining duration only.
- As a result of the inclusion/exclusion criteria, the sample size was reduced from the entire cohort.

A considerable amount of literature supports numerous medical benefits of breastfeeding for children in reducing for example, the risk of nonspecific gastroenteritis, severe lower respiratory tract infections, atopic dermatitis, obesity, and high systolic blood pressure.[1-3] However, the benefits of breastfeeding on children's behavioural outcomes are less clear-cut. Differing hypotheses have been put forth regarding potential mechanisms for the perceived associations between breastfeeding and behavioural outcomes. For example, it has been suggested that breastfeeding may lead to reduced behavioural difficulties as a result of early skin-to-skin contact, when active bonding is present, helping to promote the development of a secure bond between mother and baby.[(e.g.,4)] This may be particularly salient in protecting against the emergence of internalising behaviours as children develop.[5] On the other hand, associations between breastfeeding and behavioural outcomes may be the result of the long-chain polyunsaturated fatty acids (n-3 and n-6 PUFA) found in breast milk, which arguably impact on brain development and white growth matter; an area of the brain which is typically underdeveloped in children who display with elevated levels of externalising behaviours.[6-7] Moreover, deficits in n-3 PUFA in particular, have been shown to increase the risk of neuronal abnormalities in studies of rats, associated with increased levels of anxiety, depression, aggression, inattention and hyperactivity.[(e.g.,8-10)] While the mechanisms suggested have differing implications regarding pathways of potential effects, it is important to first be able to untangle whether 'effects' exist for children's behavioural outcomes, irrespective of selection and confounding. For example, it may be possible that there is no direct mechanism through which breastfeeding is implicated in behavioural outcomes, but rather, associations found may be an artefact of maternal and family-level characteristics.

There has been mixed support for associations between breastfeeding and externalising behaviours such as conduct problems and hyperactivity[(e.g.,11-15)], and internalising behaviours such as anxiety and depression[(e.g., 5,16-18)], in studies from infancy to adulthood. When associations are observed, a duration of four-to-six months or longer appears to be most common. Systematic differences between studies regarding definitions of breastfeeding, classification of behaviours, timing of assessment, and statistical approaches for handling confounding and selection bias, are likely contributing factors accounting for these inconsistencies. For example, self-selection into breastfeeding has repeatedly been implicated in studies examining breastfeeding and developmental outcomes. In developed countries in particular, research continues to demonstrate characteristics common in mothers who breastfeed (e.g., lower engagement in high-risk prenatal behaviours, higher education, higher income, and older age at child birth[e.g.,19-20]), which are also associated with behavioural outcomes. If associations between breastfeeding and behavioural difficulties are an artefact of maternal or family characteristics, differences in statistical approaches for handling selection bias will have important consequences. Indeed, this is reflected in the literature whereby the greater the number of implicated confounders are controlled, often, the less likely significant associations remain.[21]

On the other hand, using cohorts from developing countries may provide additional insights given the differing confounding structures.[22] For example, notable differences between developed and developing countries regarding associated maternal characteristics were recently demonstrated in the lancet series.[20] More specifically, maternal characteristics common of mothers in developing countries who breastfeed, particularly for longer durations, included poverty, lower socio-economic-status, and in some cases, lower maternal education.[23-24] Given this inverse association between socio-economic standing and selection into breastfeeding, replication of associations between breastfeeding and behavioural problems, may offer additional advantages in better understanding potential 'effects'. Currently, there are a lack of studies examining breastfeeding and behavioural outcomes using nationally representative cohorts of infants in Chile, which provides such a possibility. Additionally, challenges in examining associations between longer durations of breastfeeding on behavioural outcomes due to low prevalence rates in many developed countries is common. In Chile, duration of breastfeeding has been steadily increasing over the past decade and was reported in 2014 at 63% of mothers who were still exclusively breastfeeding when infants were six-months of age.[25] Taken together, examining breastfeeding duration and behavioural outcomes with a nationally representative cohort from Chile, may offer additional benefits in our understanding.

Objectives

To examine breastfeeding and children's behavioural outcomes using a quasi-experimental statistical technique to reduce observable differences between groups, whereby attempting to address inherent limitations in observational studies. Duration of breastfeeding was examined. Moreover, we examined whether in using a Chilean cohort, we could replicate the findings of Girard et al.[11-12] regarding reduced hyperactivity for children breastfed. We extend upon this work by also examining internalising behaviours. We hypothesised, in line with previous findings and recommendations of the WHO[26], that children who were breastfed for a minimum of six-months would present with reduced behavioural problems in early childhood.

Method

Participants included families enrolled in the "Encuesta Longitudinal de la Primera Infancia cohort, ELPI", recruited in Chile in 2010 and 2012. Families recruited in the second wave (i.e., in 2012) were not considered in this study given that child outcomes were not available longitudinally. The cohort was initially recruited to better understand the sociodemographic backgrounds of children and their families alongside their physical, social, and emotional development over time. The cohort is representative of children born between January 2006 and August 2009 in urban and rural areas, across all regions of Chile.[27] A total of 15,175 families with children between the ages of seven and 58-months were initially contacted for inclusion. At wave one, 14,161 families were assessed, 93.3% of the targeted sample. However, 487 children did not

have information pertaining to their age and were excluded. Inclusion criteria in this study were children aged seven to 24-months, who had complete data on all confounders, and who were born full term (n=4,375). Additionally, mothers who were still breastfeeding when behavioural measures were collected and who had breastfed more than six, but less than 12-months, were excluded (n=442), as it was not possible to identify whether they should be included in the group of children breastfed between seven and 12-months or in the extended breastfeeding group. This resulted in a possible sample of 3,933 children and their families, 50.6% of whom were boys (n=1,992). However, missing outcome data at wave two in 2012, resulted in a final sample 3,037. Demographic characteristics of the included children and their families can be found in Table 1. A table comparing the entire cohort to those included can be found in the online supplement. Ethical approval was obtained from the Universidad de Chile, Centro de Microdatos. Parents/guardians were provided with oral and written information about the study and informed consent was given with completion of the first questionnaire.

Children's behaviours were assessed using the Child Behaviour Checklist 1½-5 (CBCL)[28]. The CBCL 1½-5 is a parent report used to identify behaviour problems in children between the ages of one-and-a-half and five years. It is comprised of 99 items divided into subscales. The seven subscales include: emotionally reactive (9 items), anxious/depressed (8 items), somatic complaints (11 items), withdrawn (8 items), sleep problems (7 items), attention problems (5 items), and aggression (19 items). Parents rate each individual behavioural item on 3-point likert scale ranging from 0 (*not true*) to 2 (*very true or often true*), with higher scores indicative of more problematic behaviour. The CBCL 1½-5 is available in several languages, including Spanish, and has previously been validated in the literature with Chilean samples.[(e.g., 29)] The CBCL was collected at wave two, in 2012, when children were between the ages of 32 and 48-months. Means and standard deviations, along with correlations between subscales are presented in Table 2.

Breastfeeding information was collected at wave one, both retrospectively and prospectively, given the unique sampling approach of recruitment of families with children between the ages of seven and 58 months. Due to potential recall bias and in an attempt to create more homogeneity within mother's duration of recall, we included only families with children between seven and 24-months. Mothers were asked two questions: "was the newborn breastfed by his/her biological mother?" and "until what month was the child breastfed by his/her biological mother?" No information was collected regarding full breastfeeding in the cohort. Of the sample included, only 140 mothers/caregivers reported that the child had never been breastfed. Based on the WHO recommendations, we grouped children into one of four categories of duration; never breastfed (n=140), breastfed up to six complete months (n = 1,277), breastfed between seven and 12 complete months (n=1,234), and breastfed ≥ 13 months (n = 1,282). Each category of duration was treated as mutually exclusive, dummy coded, and compared against children who had never been breastfed.

Numerous confounders and self-selection into breastfeeding have been argued to account, at least in part, for previous associations between breastfeeding and behavioural outcomes. In the current study we matched groups on 13 of the most commonly identified factors, along with children's age given the variation in this sample. At the family level these included the category of the healthcare system that the family belonged to (public, private) as a proxy of income, and the presence of a partner/spouse in the household (yes/no). At the maternal level, these included maternal age (≤24 years, 25-29 years, 30-34 years, ≥35 years), educational level (no formal education, primary, secondary, vocational training, university training, postgraduate), maternal IQ (a score of 8 or below on the Wechsler Intelligence Adult Scale, digit and vocabulary scales; WAIS,[30]), working status (yes/no), type of work (professional/managerial, non-manual/skilled manual, semi-skilled/unskilled, unknown/never worked), diagnosis of depression by a healthcare professional during pregnancy (yes/no), smoking during pregnancy (yes/no), and delivery type (vaginal, caesarean). To note, the WAIS has been adapted in Chile with good reported reliability and validity.[31-32] At the child level, four confounders were included, namely, child sex (boy/girl), weight at birth (≥2500 grams, yes/no), whether the child was placed in an incubator after delivery (yes/no), and age.

Statistical Analysis

Given ethical issues with randomisation, we employed the use of propensity score matching (PSM), a statistical approach which attempts to ensure equivalence between treatment and control groups (i.e., breastfed, not breastfed), by matching groups on the most relevant factors, subsequently reducing selection bias and confounding. That is, comparisons are made between children who were breastfed and those who were not based on their measured characteristics and similar propensities for being breastfed. Nearest neighbour 1:1 models, with replacement were used. In nearest neighbour matching, groups are first randomly ordered to reduce possible bias in the matching procedure, with matching then occurring sequentially. To ensure the most optimal matches between pairs on propensity scores, we imposed a caliper of a tenth of a standard deviation. That is, for a match to occur, the propensity score of a child who was breastfed to a child who was not, had to fall within a tenth of a standard deviation of one another. Matching with replacement was necessary given the low rates of children who were never breastfed. While this technique can result in larger amounts of variance, it has been argued to reduce bias by ensuring matches are of better quality.[33] All children fell within the area of common support which refers to cases being excluded as a result of not fitting within the specified caliper. To ensure the overall quality of the matching procedure, balance checks were conducted on individual confounders and the overall models. For individual factors, remaining bias ranged between 0.0 and 18.8% and the overall mean remaining bias for models ranged between 5.5% and 7.2%. It has been suggested that less than 20% remaining bias is indicative of good matching,[34] thus we concluded that our matching was successful. We report on the average treatment effects of those treated (ATT). All

analyses were conducted using Stata v14 software. We use the term significant henceforth to denote statistical significance.

Results

Comparing children never breastfed to those who were breastfed up to six months inclusive, significant differences in favour of those who were breastfed were found on two of the behavioural subscales (i.e., emotionally reactive and somatic complaints). These results remained significant following matching and were in the expected direction whereby children who were breastfed had lower scores on these subscales (i.e., a mean difference of -1.05 and -1.26, respectively). Comparing children who were never breastfed to those breastfed between seven and 12 months inclusive, significant differences were found prior to matching on all subscales with the exception of anxious/depressed and sleep problems. After matching, significant differences remained for emotional reactivity, somatic complaints, and attention problems (i.e., a mean difference of -1.05, -0.83 and -0.52, respectively). Once again results were in the expected direction with reduced difficulties for children who were breastfed. Comparing those never breastfed to the extended group (i.e., 13 months or more), pre-matching results were similar to the first model with lower scores on the emotionally reactive and somatic complaints subscales. Post matching revealed no remaining significant differences between groups. All results can be found in Table 3.

Discussion

While causality remains an ongoing debate, owing to the ethical constraints of conducting RCTs, our results add to the emerging corpus of literature trying to untangle the 'effects' of breastfeeding on behavioural outcomes by using a PSM approach in a Chilean cohort. Given the knowledge of family and maternal characteristics commonly observed of children who are breastfed, the use of matching helps to ensure that differences between groups on these observable characteristics are significantly minimised, so that the propensity to be breastfed across groups is arguably comparable. As a result, only post-matching results are discussed. Due to exclusions, our results have implications only for children who were born full term.

The results suggest that breastfeeding may be an effective low-cost early investment for reducing difficulties with emotional reactivity and somatic complaints, in addition to attention problems experienced in early childhood; all of which are indicative of neurodevelopmental difficulties. Duration appears to be an important factor implicated in these associations. For example, results revealed that any breastfeeding during the first six months, and up to 12 full months, contributed to reductions in children's difficulties with emotional reactivity and somatic complaints up to four years of age. In comparison, a minimum of at least six full months or more of breastfeeding was required for observed benefits related to attention problems at the same age. This latter finding is in line with findings from Girard et al.,[11], who also used PSM in a nationally

representative sample of infants from Ireland. The similar results found around three years of age, across these two unique cohorts, whilst using PSM, demonstrates favourable support for a direct mechanism between breastfeeding and reduced attention problems in early childhood, rather than confounding per se. Worth noting is the link found between inattention/hyperactivity, regulation difficulties and mood disorders[35], and their link with deficiencies in arachidonic acid and docosahexaenoic acid.[36] Given the types of behaviours where reductions for those breastfed are found in this study, and in the context of previous studies, a plausible hypothesis might be that of the nutrients found in breastmilk contributing to the growing infant's brain development. More research in this area, using well designed and rigorously sound methodology is first needed before firm conclusions can be drawn.

Conversely, no benefits of extended breastfeeding were found on children's internalising or externalising behavioural problems. This may suggest a non-linear dose-response effect of breastfeeding on behaviour, similar to previous findings. [(e.g.,12)] While the recommendations put forth by the WHO for continued partial breastfeeding up to two years of age or more for the physical health and growth of infants has been suggested, we observed no additional benefits of extended breastfeeding on behavioural problems in this sample. These findings do not however contradict these recommendations regarding the many afforded medical benefits of extended durations of breastfeeding. Of interest and as can be seen in Table 1, mothers who breastfed for extended durations in Chile had similar characteristics to mothers who had never breastfed. For example, in both the never- and extended breastfeeding groups, a significantly higher proportion of mothers had never worked, were in the public tier of the health system, had only completed education at the primary level and had below average scores on both the digit and vocabulary scales of the WAIS. While a non-linear dose-response hypothesis is plausible, these findings also likely support what is already known. Maternal characteristics contribute to children's behavioural outcomes and the nutrients alone found in breastmilk may be only part of the story. Moreover, it supports the differences in confounding structures of breastfeeding in developing and developed countries. [20,22]

Notable strengths of this study include the use of the largest nationally representative cohort of Chilean children to date, where self-selection and confounding structures differ from developed countries, whilst utilising PSM, with a large number of matching confounders to reduce differences across groups, of which included maternal IQ. Despite these strengths, notable limitations must be mentioned. While information on necessary supplementation was asked, there was no specific information regarding full breastfeeding, limiting our ability to examine its impact. This is an important issue given the differences in feeding experiences and the potential for dilution of effects from breastfeeding to behaviour. In the same vein, no information regarding direct breastfeeding versus expressed breast milk was collected; information which may help in better understanding pathways of effect. Due to our inclusion/exclusion criteria, the sample size was significantly reduced, with some statistically significant differences between the originally

recruited cohort and those included in the current study. Additionally, despite the benefits afforded by PSM techniques, matching is only possible on observable characteristics. While we were able to include a multitude of health and social confounders, including maternal IQ, it remains possible that unobservable characteristics contribute to the associations. Finally, shared method variance is a concern given that parent reports were used to collect information on both breastfeeding duration and child behaviours.

Despite these limitations, and in the context of the strengths of this study, we believe these results contribute important findings, namely more support for the potential of 'causal paths'. While a comprehensive answer to the question of effects on psychosocial development remains unanswered, with replication across regions whilst using more stringent methodological approaches to help in reducing bias inherent in observational studies, promise for better understanding of potential mechanisms is viable.

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Table 1: *Family, maternal, infant, and medical characteristics: Infant cohort between 7 and 24 months*

	Never breastfeed (n=140)	1-6 months (n=1277)	7-12 months (n=1234)	13 months or more (n=1282)	*p*
	n (%)	**n (%)**	**n (%)**	**n (%)**	
Resident spouse/partner (yes)	90 (64.3)	851 (66.6)	859 (69.6)	891 (69.5)	*ns*
Social class					≤ .001
Professional/managerial	8 (5.7)	111 (8.7)	98 (7.9)	116 (9.0)	
Non-manual/skilled manual	50 (35.7)	512 (40.1)	487 (39.5)	355 (27.7)	
Semiskilled/unskilled	5 (3.6)	33 (2.6)	23 (1.9)	34 (2.7)	
Never worked	77 (55.0)	621 (48.6)	626 (50.7)	777 (60.6)	
Health provisional system					≤ .001
Public system	127 (90.7)	1107 (86.7)	1069 (86.6)	1205 (94.0)	
Private system	13 (9.3)	170 (13.3)	165 (13.4)	77 (6.0)	
Maternal education					≤ .001
No formal education	0 (0)	3 (0.2)	4 (0.3)	7 (0.5)	
Primary complete	26 (18.6)	155 (12.1)	154 (12.5)	279 (21.8)	
Secondary complete	59 (42.1)	491 (38.4)	483 (39.1)	544 (42.4)	
Vocational training	42 (30.0)	441 (34.5)	392 (31.8)	347 (27.1)	
University training	13 (9.3)	163 (12.8)	176 (14.3)	86 (6.7)	
Postgraduates studies	0 (0)	13 (1.0)	17 (1.4)	7 (0.5)	
Unknown	0 (0)	11 (0.9)	8 (0.6)	12 (0.9)	
Maternal working status (yes)	51 (36.4)	533 (41.7)	505 (40.9)	407 (31.7)	≤ .001
Maternal age					≤ .001
≤ 24	66 (47.1)	524 (41.0)	425 (34.4)	485 (37.8)	
25-29	21 (15.0)	294 (23.0)	311 (25.2)	258 (20.1)	
30-34	27 (19.3)	239 (18.7)	284 (23.0)	265 (20.7)	
≥ 35	26 (18.6)	220 (17.2)	214 (17.3)	274 (21.4)	
Maternal IQ					
WAIS digit (below average)	103 (73.6)	825 (64.6)	768 (62.2)	918 (71.6)	≤ .001
WAIS vocabulary (below average)	73 (52.1)	534 (41.8)	502 (40.7)	671 (52.3)	≤ .001
Maternal depression during pregnancy (yes)	23 (16.4)	178 (13.9)	185 (15.0)	188 (14.7)	*ns*
Smoking during pregnancy (yes)	19 (13.6)	141 (11.0)	101 (8.2)	151 (11.8)	.011
Alcohol use during pregnancy (yes)	14 (10.0)	96 (7.5)	77 (6.2)	96 (7.5)	*ns*
Drugs use during pregnancy (yes)	4 (2.9)	7 (0.5)	8 (0.6)	10 (0.8)	.025
Delivery mode (caesarean)	72 (51.4)	606 (47.5)	578 (46.8)	503 (39.2)	≤ .001
Birth weight (≥2500g, yes)	11 (7.9)	34 (2.7)	30 (2.4)	28 (2.2)	.001
Stay in incubator (yes)	12 (8.6)	46 (3.6)	44 (3.6)	49 (3.8)	.030
Infant sex (boy)	67 (47.9)	658 (51.5)	637 (51.6)	630 (49.1)	*ns*

Note: The health provisional system was used as a proxy for the financial status of the family, whereby families in the private system are generally of higher income. Maternal IQ was assessed using both the digit and vocabulary scales of the Wechsler Intelligence Adult Scale, WAIS (Wechsler, 1939). A below average score is defined as a score of 8 or below on the digit and vocabulary scales.

Table 2: *Bivariate Correlations and Means (Standard Deviations) of Children's Behaviours*

	Emotionally reactive	Anxious/ depressed	Somatic complaints	Withdrawn	Sleep problems	Attention problems	Aggressive
Emotionally reactive							
Anxious/depressed	.650**						
Somatic complaints	.549**	.499**					
Withdrawn	.569**	.548**	.435**				
Sleep problems	.445**	.404**	.373**	.350**			
Attention problems	.428**	.374**	.319**	.345**	.341**		
Aggressive	.664**	.542**	.409**	.496**	.477**	.619**	
Mean	3.11	4.33	3.10	2.92	2.83	3.72	13.68
Standard deviation	2.94	3.01	2.63	2.38	2.62	1.97	8.35

Note: ** denotes statistical significance at the 0.01 level

Table 3: *Breastfeeding and Children's Behavioural Problems: Pre and Post Matching Results*

Up to 6 months	Pre Matching				Post Matching			
	T	C	Diff (Sig.)	S.E	T	C	Diff (Sig.)	S.E
Emotionally reactive	3.16	3.98	**-0.81****	0.30	3.16	4.22	**-1.05***	0.43
Anxious/depressed	4.38	4.67	-0.29	0.29	4.38	4.28	-0.09	0.39
Somatic complaints	3.09	3.73	**-0.64***	0.25	3.09	4.35	**-1.26****	0.38
Withdrawn	3.00	3.16	-0.15	0.24	3.00	3.20	-0.20	0.30
Sleep problems	2.86	3.07	-0.20	0.26	2.86	3.21	-0.35	0.35
Attention problems	3.79	4.05	-0.26	0.19	3.79	4.04	-0.25	0.22
Aggression	13.91	14.90	-0.98	0.85	13.91	14.52	-0.61	1.17
Between 7 and 12 months								
Emotionally reactive	2.85	3.98	**-1.12*****	0.29	2.85	3.91	**-1.05****	0.43
Anxious/depressed	4.12	4.67	-0.54	0.30	4.12	4.36	-0.23	0.38
Somatic complaints	2.95	3.73	**-0.78****	0.26	2.95	3.78	**-0.83***	0.37
Withdrawn	2.70	3.16	**-0.45***	0.22	2.70	3.12	-0.41	0.29
Sleep problems	2.68	3.07	-0.38	0.26	2.68	3.05	-0.37	0.36
Attention problems	3.57	4.05	**-0.48***	0.19	3.57	4.09	**-0.51***	0.23
Aggression	13.02	14.90	**-1.87***	0.81	13.02	14.83	-1.81	1.21
13 months or more								
Emotionally reactive	3.20	3.98	**-0.77***	0.30	3.20	3.57	-0.37	0.45
Anxious/depressed	4.42	4.67	-0.24	0.31	4.42	4.42	0.00	0.43
Somatic complaints	3.18	3.73	**-0.55***	0.27	3.18	3.83	-0.65	0.41
Withdrawn	3.01	3.16	-0.15	0.24	3.01	2.86	0.15	0.32
Sleep problems	2.92	3.07	-0.15	0.27	2.92	2.62	0.29	0.39
Attention problems	3.76	4.05	-0.29	0.20	3.76	3.89	-0.13	0.24
Aggression	13.91	14.90	-0.98	0.86	13.91	12.88	1.02	1.33

Note: *** denotes significance at the p = < .001 level, ** at the .01 level, * at the .05 level. T denotes 'treatment' (breastfed) and C denotes 'control' (not breastfed). 'Diff' represents the difference in scores between groups. S.E. refers to the standard errors. For being breastfed up to 6 months: N for the treatment group was 949 and 110 for the control group. For being breastfed between 7 and 12 months: N for the treatment group was 946 and 110 for the control group. For being breastfed 13 months or more: N for the treatment group was 1,006 and 110 for the control group.

Online Supplement 1

Family, maternal, infant, and medical characteristics: A comparison between the Entire and Included Cohort

	Entire Cohort N = 14,161 (%)	**Included Families N = 3,933 (%)**	***p***
Resident spouse/partner (yes)	6,873 (69.2%)	2,691 (68.4%)	*ns*
Social class			≤ .001
Professional/managerial	968 (9.7%)	333 (8.5%)	
Non-manual/skilled manual	3,812 (38.4%)	1,404 (35.7%)	
Semi-skilled/unskilled	374 (3.8%)	95 (2.4%)	
Never worked	4,779 (48.1%)	2,101 (53.4%)	
Health provisional system			*ns*
Public system	8,820 (88.8%)	3,508 (89.2%)	
Private system	1,113 (11.2%)	425 (10.8%)	
Maternal education			*ns*
No formal education	37 (0.4%)	14 (0.4%)	
Primary complete	1,743 (17.7%)	614 (15.7%)	
Secondary Complete	3,917 (39.8%)	1,577 (40.4%)	
Vocational training	2,984 (30.3%)	1,222 (31.3%)	
University training	1,075 (10.9%)	438 (11.2%)	
Postgraduate studies	81 (0.8%)	37 (0.9%)	
Maternal working status (yes)	4,366 (44.0%)	1,496 (38.0%)	≤ .001
Maternal age			≤ .001
≤ 24	2,806 (28.2%)	1,500 (38.1%)	
25-29	2,250 (22.7%)	884 (22.5%)	
30-34	2,173 (21.9%)	815 (20.7%)	
≥ 35	2,704 (27.2%)	734 (18.7%)	
Maternal depression during pregnancy (yes)	1,027 (10.3%)	574 (14.6%)	≤ .001
Smoking during pregnancy (yes)	978 (9.9%)	412 (10.5%)	*ns*
Alcohol use during pregnancy (yes)	705 (7.1%)	283 (7.2%)	*ns*
Drug use during pregnancy (yes)	81 (0.8%)	29 (0.7%)	*ns*
Delivery mode (caesarean)	4,314 (43.5%)	1,759 (44.7%)	*ns*
Birth weight (≥ 2500g, yes)	205 (2.3%)	103 (2.6%)	*ns*
Stay in incubator (yes)	433 (4.4%)	151 (3.8%)	*ns*
Infant sex (boy)	5,004 (50.4%)	1,992 (50.6%)	*ns*

BMJ Open

Breastfeeding and behavioural problems: Propensity score matching with a national cohort of infants in Chile

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-025058.R1
Article Type:	Research
Date Submitted by the Author:	03-Oct-2018
Complete List of Authors:	Lisa-Christine , Girard; University of Edinburgh , Clinical Psychology Farkas, Chamarrita; Pontificia Universidad Catolica de Chile, Psychology
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Paediatrics, Nutrition and metabolism
Keywords:	Breastfeeding, Behaviour Problems, Cohort Study

Breastfeeding and behavioural problems: Propensity score matching with a national cohort of infants in Chile

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Short title: Breastfeeding and behaviour

Abbreviations: Encuesta Longitudinal de la Primera Infancia cohort, ELPI; Propensity Score Matching PSM; Child Behaviour Checklist CBCL; World Health Organization WHO; Randomized Control Trial RCT; Attention to Treat ATT

Author Contributions:
Dr. Girard and Prof. Farkas conceptualized the study, carried out the analyses, interpreted the data, drafted the initial manuscript, critically reviewed and revised the manuscript, and approved the final manuscript as submitted. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Data Access, Responsibility, and Analysis: Dr. Girard and Prof. Farkas had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Data Sharing Statement:
Data is not able to be deposited in an open repository however researchers wishing to access the data may do so directly by writing a request to the Centro de Microdatos de la Facultad de Economía y Negocios de la Universidad de Chile.

Conflict of Interest Statement: The authors have no conflicts of interest relevant to this article to disclose.

Financial Disclosure Statement: The authors have no financial relationships relevant to this article to disclose.

Word Count: 3000

Key words: Breastfeeding; Behaviour Problems; Propensity Score Matching; Epidemiology; Developmental Cohort

Abstract

Importance: Potential effects of breastfeeding on children's behaviour remains an elusive debate given inherent methodological challenges. Propensity score matching affords benefits by ensuring greater equivalence on observable social and health determinants, helping to reduce bias between groups.

Objectives: We examined whether duration of breastfeeding had an impact on children's externalising and internalising behaviours.

Study Design: A cohort study (Encuesta Longitudinal de la Primera Infancia cohort) that included 3,037 Chilean families who were enrolled in 2010. Follow-up data was collected in 2012.

Setting: General community.

Participants: Population-based sample. Eligibility criteria: children born full-term with complete data on matching variables. Matching variables included: healthcare system as a proxy of income, presence of a partner/spouse in the household, maternal age, educational level, IQ, working status, type of work, diagnosis of prenatal depression by a healthcare professional, smoking during pregnancy, delivery type, child sex, weight at birth, incubation following delivery, and child age.

Exposure: Duration of breastfeeding.

Main Outcomes and Measures: Externalising and internalising problems assessed using the Child Behaviour Checklist.

Results: Matched results revealed benefits of any breastfeeding, up to six months, on emotional reactivity and somatic complaints, (mean difference of -1.00, 95% CI, -1.84 to -0.16 and -1.02, 95% CI, -1.76 to -0.28, respectively). Children breastfed between 7-12 months also had reduced scores on emotional reactivity, in addition to attention problems (mean difference of -0.86, 95% CI, -1.66 to -0.06 and -0.50, 95% CI, -0.93 to -0.07, respectively). No benefits were observed for children breastfed 13 months or more.

Conclusion: Reduced internalising difficulties and inattention were found in children breastfed up to a year, suggesting that breastfeeding may have beneficial impacts on these areas of development. The magnitude of effect was modest. Extended durations of breastfeeding did not appear to offer any benefits.

Article Summary:

Strengths:

- Use of a quasi-experimental statistical approach to match children with the propensity to be breastfed to those who were not on observable health and social determinates.
- Use of a large Chilean cohort where confounding structure differs from developed countries.
- The inclusion of 14 matching variables including maternal IQ, which is almost double the average amount of variables included in similar studies.

Limitations:

- No specific information was collected on full breastfeeding in this cohort restricting the study to examining duration only.
- As a result of the inclusion/exclusion criteria, the sample size was reduced from the entire cohort.

Introduction

A considerable amount of literature supports numerous medical benefits of breastfeeding for children in reducing for example, the risk of nonspecific gastroenteritis, severe lower respiratory tract infections, atopic dermatitis, obesity, and high systolic blood pressure.[1-3] However, the benefits of breastfeeding on children's behavioural outcomes are less clear-cut. Differing hypotheses have been put forth regarding potential mechanisms for the perceived associations between breastfeeding and behavioural outcomes. For example, it has been suggested that breastfeeding may lead to reduced behavioural difficulties as a result of early skin-to-skin contact, when active bonding is present, helping to promote the development of a secure bond between mother and baby.[(e.g.,4)] This may be particularly salient in protecting against the emergence of internalising behaviours as children develop.[5] On the other hand, associations between breastfeeding and behavioural outcomes may be the result of the long-chain polyunsaturated fatty acids (n-3 and n-6 PUFA) found in breast milk, which arguably impact on brain development and white growth matter; an area of the brain which is typically underdeveloped in children who display with elevated levels of externalising behaviours.[6-7] Moreover, deficits in n-3 PUFA in particular, have been shown to increase the risk of neuronal abnormalities in studies of rats, associated with increased levels of anxiety, depression, aggression, inattention and hyperactivity.[(e.g.,8-10)] While the mechanisms suggested have differing implications regarding pathways of potential effects, it is important to first be able to untangle whether 'effects' exist for children's behavioural outcomes, irrespective of selection and confounding. For example, it may be possible that there is no direct mechanism through which breastfeeding is implicated in behavioural outcomes, but rather, associations found may be an artefact of maternal and family-level characteristics.

There has been mixed support for associations between breastfeeding and externalising behaviours such as conduct problems and hyperactivity[(e.g.,11-15)], and internalising behaviours such as anxiety and depression[(e.g., 5,16-18)], in studies from infancy to adulthood. When associations are observed, a duration of four-to-six months or longer appears to be most common. Systematic differences between studies regarding definitions of breastfeeding, classification of behaviours, timing of assessment, and statistical approaches for handling confounding and selection bias, are likely contributing factors accounting for these inconsistencies. For example, self-selection into breastfeeding has repeatedly been implicated in studies examining breastfeeding and developmental outcomes. In developed countries in particular, research continues to demonstrate characteristics common in mothers who breastfeed (e.g., lower engagement in high-risk prenatal behaviours, higher education, higher income, and older age at child birth[e.g.,19-20]), which are also associated with behavioural outcomes. If associations between breastfeeding and behavioural difficulties are an artefact of maternal or family characteristics, differences in statistical approaches for handling selection bias will have important consequences. Indeed, this is reflected in the literature whereby the greater the number of implicated confounders are controlled, often, the less likely significant associations remain.[21]

On the other hand, using cohorts from developing countries may provide additional insights given the differing confounding structures.[22] For example, notable differences between developed and developing countries regarding associated maternal characteristics were recently demonstrated in the Lancet series.[20] More specifically, maternal characteristics common of mothers in developing countries who breastfeed, particularly for longer durations, included poverty, lower socio-economic-status, and in some cases, lower maternal education.[23-24] Given this inverse association between socio-economic standing and selection into breastfeeding, replication of associations between breastfeeding and behavioural problems, may offer additional advantages in better understanding potential 'effects'. Currently, there are a lack of studies examining breastfeeding and behavioural outcomes using nationally representative cohorts of infants in Chile, which provides such a possibility. While economic growth has been observed, social inequalities in Chile remain high, particularly for women[25]. Additionally, challenges in examining associations between longer durations of breastfeeding on behavioural outcomes due to low prevalence rates in many developed countries is common. In Chile, duration of breastfeeding has been steadily increasing over the past decade and was reported in 2014 at 63% of mothers who were still exclusively breastfeeding when infants were six-months of age.[26] Taken together, examining breastfeeding duration and behavioural outcomes with a nationally representative cohort from Chile, may offer additional benefits in our understanding.

Objectives

To examine breastfeeding and children's behavioural outcomes longitudinally, using a quasi-experimental statistical technique to reduce observable differences between groups, whereby attempting to address inherent limitations in observational studies. Duration of breastfeeding was examined. Moreover, we examined whether in using a Chilean cohort, we could replicate the findings of Girard et al.[11-12] regarding reduced hyperactivity for children breastfed, following propensity score matching, in two separate longitudinal Irish cohorts. We extend upon this work by also examining internalising behaviours. We hypothesised, in line with previous findings and recommendations of the WHO[27], that children who were breastfed for a minimum of six-months would present with reduced behavioural problems in early childhood.

Method

Participants included families enrolled in the "Encuesta Longitudinal de la Primera Infancia cohort, ELPI", recruited in Chile in 2010 and 2012. Families recruited in the second wave (i.e., in 2012) were not considered in this study given that child outcomes were not available longitudinally. The cohort was initially recruited to better understand the sociodemographic backgrounds of children and their families alongside their physical, social, and emotional development over time. The cohort is representative of children born between January 2006 and August 2009 in urban and rural areas, across all regions of Chile.[28] A total of

15,175 families with children between the ages of seven and 58-months were initially contacted for inclusion. At wave one, 14,161 families were assessed, 93.3% of the targeted sample. However, 487 children did not have information pertaining to their age and were excluded. Inclusion criteria in this study were children aged seven to 24-months, who had complete data on all confounders at wave one, and who were born full term (n=4,375). Additionally, mothers who were still breastfeeding at wave one who had breastfed more than six, but less than 12-months, were excluded (n=442), as it was not possible to identify whether they should be included in the group of children breastfed between seven and 12-months or in the extended breastfeeding group. This resulted in a possible sample of 3,933 children and their families, 50.6% of whom were boys (n=1,992) at wave one. However, missing outcome data (i.e., child behaviours) at wave two in 2012, resulted in a final sample 3,037. Demographic characteristics of the included children and their families can be found in Table 1. A table comparing the entire cohort to those included can be found in the online supplement. Ethical approval was obtained from the Universidad de Chile, Centro de Microdatos. Parents/guardians were provided with oral and written information about the study and informed consent was given with completion of the first questionnaire.

Children's behaviours were assessed using the Child Behaviour Checklist 1½-5 (CBCL)[29]. The CBCL 1½-5 is a parent report used to identify behaviour problems in children between the ages of one-and-a-half and five years. It is comprised of 99 items divided into subscales. The seven subscales include: emotionally reactive (9 items), anxious/depressed (8 items), somatic complaints (11 items), withdrawn (8 items), sleep problems (7 items), attention problems (5 items), and aggression (19 items). Parents rate each individual behavioural item on 3-point Likert scale ranging from 0 (*not true*) to 2 (*very true or often true*), with higher scores indicative of more problematic behaviour. The CBCL 1½-5 is available in several languages, including Spanish, and has previously been validated in the literature with Chilean samples.[(e.g., 30)] The CBCL was collected at wave two, in 2012, when children were between the ages of 32 and 48-months. Means and standard deviations, along with correlations between subscales are presented in Table 2.

Breastfeeding information was collected at wave one, both retrospectively and prospectively, given the unique sampling approach of recruitment of families with children between the ages of seven and 58 months. Due to potential recall bias and in an attempt to create more homogeneity within mother's duration of recall, we included only families with children between seven and 24-months. Mothers were asked two questions: "was the newborn breastfed by his/her biological mother?" and "until what month was the child breastfed by his/her biological mother?" No information was collected regarding full breastfeeding in the cohort. Of the sample included, only 140 mothers/caregivers reported that the child had never been breastfed. Based on the WHO recommendations, we grouped children into one of four categories of duration; never breastfed (*n*=140), breastfed up to six complete months (*n* = 1,277), breastfed between seven and 12

complete months (n=1,234), and breastfed ≥ 13 months (n = 1,282). Each category of duration was treated as mutually exclusive, dummy coded, and compared against children who had never been breastfed.

Numerous confounders and self-selection into breastfeeding have been argued to account, at least in part, for previous associations between breastfeeding and behavioural outcomes. In the current study we matched groups on 13 of the most commonly identified factors, along with children's age given the variation in this sample. At the family level these included the category of the healthcare system that the family belonged to (public, private) as a proxy of income, and the presence of a partner/spouse in the household (yes/no). At the maternal level, these included maternal age, educational level (no formal education, primary, secondary, vocational training, university training, postgraduate), maternal IQ (a score of 8 or below on the Wechsler Intelligence Adult Scale, digit and vocabulary scales; WAIS,[31]), working status (yes/no), type of work (professional/managerial, non-manual/skilled manual, semi-skilled/unskilled, unknown/never worked), diagnosis of depression by a healthcare professional during pregnancy (yes/no), smoking during pregnancy (yes/no), and delivery type (vaginal, caesarean). To note, the WAIS has been adapted in Chile with good reported reliability and validity.[32-33] At the child level, four confounders were included, namely, child sex (boy/girl), weight at birth (≥2500 grams, yes/no), whether the child was placed in an incubator after delivery (yes/no), and age at first assessment in wave 1.

Patient and Public Involvement

The development of the research question and outcome measures, along with study design and recruitment to, were not directly informed by patients' priorities, experience or preference. Study findings will be disseminated to the Ministry of Labor and Social Welfare, whom were responsible for waves 1 and 2, and the Ministry of Social Development, who is currently responsible for wave 3 of the ELPI cohort, ensuring greater likelihood of dissemination to study participants.

Statistical Analysis

We employed the use of propensity score matching (PSM), a statistical approach which attempts to ensure equivalence between treatment and control groups (i.e., breastfed, not breastfed), by matching groups on the most relevant factors, subsequently reducing selection bias and confounding. That is, comparisons are made between children who were breastfed and those who were not based on their measured characteristics and similar propensities for being breastfed. Nearest neighbour 1:1 models, with replacement were used. In nearest neighbour matching, groups are first randomly ordered to reduce possible bias in the matching procedure, with matching then occurring sequentially. To ensure the most optimal matches between pairs on propensity scores, we imposed a caliper of a tenth of a standard deviation. That is, for a match to occur, the propensity score of a child who was breastfed to a child who was not, had to fall within a tenth of a standard deviation of one another. Matching with replacement was necessary given the low rates of children who were never breastfed. While this technique can result in larger amounts of variance, it has been argued to reduce

bias by ensuring matches are of better quality.[34] All children fell within the area of common support which refers to cases being excluded as a result of not fitting within the specified caliper. See Figure 1 for the overlapping support of the distribution of propensity scores. To ensure the overall quality of the matching procedure, balance checks were conducted on individual confounders and the overall models. For individual factors, remaining bias ranged between 0.0 and 18.8% (see Figure 2) and the overall mean remaining bias for models ranged between 5.5% and 8.3%. It has been suggested that less than 20% remaining bias is indicative of good matching,[35] thus we concluded that our matching was successful. We report on the average treatment effects of those treated (ATT). All analyses were conducted using Stata v14 software. We use the term significant henceforth to denote statistical significance, using a threshold of $p = <.05$.

Results

Comparing children never breastfed to those who were breastfed up to six months inclusive, significant differences in favour of those who were breastfed were found on two of the behavioural subscales (i.e., emotionally reactive and somatic complaints). These results remained significant following matching whereby children who were breastfed had lower scores on these subscales (i.e., a mean difference of -1.00, d = -0.23 and -1.02, d = -0.27 respectively). Comparing children who were never breastfed to those breastfed between seven and 12 months inclusive, significant differences were found prior to matching on all subscales with the exception of anxious/depressed and sleep problems. After matching, significant differences remained for emotional reactivity and attention problems only (i.e., a mean difference of -0.86, d = -0.21 and -0.50, d = -0.22, respectively), with reduced difficulties for children who were breastfed. Comparing those never breastfed to the extended group (i.e., 13 months or more), pre-matching results were similar to the first model with lower scores on the emotionally reactive and somatic complaints subscales. Post matching revealed no remaining significant differences between groups. All results can be found in Table 3.

Discussion

While causality remains an ongoing debate, owing to the ethical constraints of conducting RCTs, our results add to the emerging corpus of literature trying to untangle the 'effects' of breastfeeding on behavioural outcomes by using a PSM approach in a Chilean cohort. Given the knowledge of family and maternal characteristics commonly observed of children who are breastfed, the use of matching helps to ensure that differences between groups on these observable characteristics are significantly minimised, so that the propensity to be breastfed across groups is arguably comparable. As a result, only post-matching results are discussed. Due to exclusions, our results have implications only for children who were born full term.

The results suggest that breastfeeding may be an effective low-cost early investment for reducing difficulties with emotional reactivity and somatic complaints, in addition to attention problems experienced

in early childhood; all of which are indicative of neurodevelopmental difficulties. Duration appears to be an important factor implicated in these associations. For example, results revealed that any breastfeeding during the first six months, and up to 12 full months, contributed to reductions in children's difficulties with emotional reactivity and somatic complaints up to four years of age. In comparison, a minimum of at least six full months or more of breastfeeding was required for observed benefits related to attention problems at the same age. This latter finding is in line with findings from Girard et al.,[11], who also used PSM in a nationally representative sample of infants from Ireland. The similar results found around three years of age, across these two unique cohorts, whilst using PSM, demonstrates favourable support for a direct mechanism between breastfeeding and reduced attention problems in early childhood, rather than confounding per se. Worth noting is the link found between inattention/hyperactivity, regulation difficulties and mood disorders[36], and their link with deficiencies in arachidonic acid and docosahexaenoic acid.[37] Given the types of behaviours where reductions for those breastfed are found in this study, and in the context of previous studies [(e.g.,8-10, 36-37)], a plausible hypothesis might be that of the nutrients found in breastmilk contributing to the growing infant's brain development. More research in this area, using well designed and rigorously sound methodology is first needed before firm conclusions can be drawn.

While our results suggest statistically significant differences in favour of children who were breastfed at least six full months (and up until 12 full months), as compared to those who were never breastfed on emotional reactivity, somatic complaints, and inattention, the magnitude of effect for each behaviour was found to be small (i.e., Cohen's $d = < .30$). The practical and clinical significance of our results is arguably interpretable in the eye of the 'stakeholder'. A small reduction in a child's emotional reactivity, somatic complaints, and/or inattention in everyday situations may carry greater importance to a first-time or multiparous mother experiencing high levels of stress and fatigue as a result of limited financial and/or personal resources. On the other hand, within a clinical context, the effect sizes found may be perceived as carrying less practical importance.

Conversely, no benefits of extended breastfeeding were found on children's internalising or externalising behavioural problems. This may suggest a non-linear dose-response effect of breastfeeding on behaviour, similar to previous findings. [(e.g.,12)] While the recommendations put forth by the WHO for continued partial breastfeeding up to two years of age or more for the physical health and growth of infants has been suggested, we observed no additional benefits of extended breastfeeding on behavioural problems in this sample. These findings do not however contradict these recommendations regarding the many afforded medical benefits of extended durations of breastfeeding. Of interest and as can be seen in Table 1, mothers who breastfed for extended durations in Chile had similar characteristics to mothers who had never breastfed, lending to poorer quality matching. For example, in both the never- and extended breastfeeding groups, a significantly higher proportion of mothers had never worked, were in the public tier of the health system, had

only completed education at the primary level and had below average scores on both the digit and vocabulary scales of the WAIS; factors which when previously controlled, have reduced observed associations between breastfeeding and children's cognitive and behavioural development outcomes. While a non-linear dose-response hypothesis is plausible, these findings also likely support what is already known. Maternal characteristics contribute to children's behavioural outcomes and the nutrients alone found in breastmilk may be only part of the story. Moreover, it supports the differences in confounding structures of breastfeeding in developing and developed countries. [20,22]

Notable strengths of this study include the use of the largest nationally representative cohort of Chilean children to date, where self-selection and confounding structures differ from developed countries, whilst utilising PSM, with a large number of matching confounders to reduce differences across groups, of which included maternal IQ. Despite these strengths, notable limitations must be mentioned. While information on necessary supplementation was asked, there was no specific information regarding full breastfeeding, limiting our ability to examine its impact. This is an important issue given the differences in feeding experiences and the potential for dilution of effects from breastfeeding to behaviour. In the same vein, no information regarding direct breastfeeding versus expressed breast milk was collected; information which may help in better understanding pathways of effect. Due to our inclusion/exclusion criteria, the sample size was significantly reduced, with some statistically significant differences between the originally recruited cohort and those included in the current study, indicative of potential selection bias. Thus, warranting replication. Additionally, despite the benefits afforded by PSM techniques, matching is only possible on observable characteristics. While we were able to include a multitude of health and social confounders, including maternal IQ, it remains possible that unobservable characteristics contribute to the associations. Relatedly, the quality of matching for the extended breastfeeding families as compared to the never breastfeeding families was not as successful compared to the matching between the other groups, due to the initial similarities on health and social factors. The included covariates used for matching were theoretically motivated and thus, we kept the integrity of matching variables intact across all models. However, the findings from this model (i.e., the extended breastfeeding families) warrants caution in interpretation. Future studies are needed to more carefully evaluate extended breastfeeding and potential associations with behavioural outcomes, in the context of differing confounding structure. Finally, shared method variance is a concern given that parent reports were used to collect information on both breastfeeding duration and child behaviours.

Despite these limitations, and in the context of the strengths of this study, we believe these results contribute important findings, namely more support for the potential of 'causal paths'. A comprehensive answer to the question of effects on psychosocial development remains unanswered without the use of RCTs. However, with replication across regions, whilst using more stringent methodological approaches to help in

reducing bias inherent in observational studies, promise for better understanding of potential mechanisms is viable.

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Table 1: *Family, maternal, infant, and medical characteristics: Infant cohort between 7 and 24 months*

	Never breastfeed (n=140)	1-6 months (n=1277)	7-12 months (n=1234)	13 months or more (n=1282)	*p*
	n (%)	**n (%)**	**n (%)**	**n (%)**	
Resident spouse/partner (yes)	90 (64.3)	851 (66.6)	859 (69.6)	891 (69.5)	*ns*
Social class					≤ .001
Professional/managerial	8 (5.7)	111 (8.7)	98 (7.9)	116 (9.0)	
Non-manual/skilled manual	50 (35.7)	512 (40.1)	487 (39.5)	355 (27.7)	
Semiskilled/unskilled	5 (3.6)	33 (2.6)	23 (1.9)	34 (2.7)	
Never worked	77 (55.0)	621 (48.6)	626 (50.7)	777 (60.6)	
Health provisional system					≤ .001
Public system	127 (90.7)	1107 (86.7)	1069 (86.6)	1205 (94.0)	
Private system	13 (9.3)	170 (13.3)	165 (13.4)	77 (6.0)	
Maternal education					≤ .001
No formal education	0 (0)	3 (0.2)	4 (0.3)	7 (0.5)	
Primary complete	26 (18.6)	155 (12.1)	154 (12.5)	279 (21.8)	
Secondary complete	59 (42.1)	491 (38.4)	483 (39.1)	544 (42.4)	
Vocational training	42 (30.0)	441 (34.5)	392 (31.8)	347 (27.1)	
University training	13 (9.3)	163 (12.8)	176 (14.3)	86 (6.7)	
Postgraduates studies	0 (0)	13 (1.0)	17 (1.4)	7 (0.5)	
Unknown	0 (0)	11 (0.9)	8 (0.6)	12 (0.9)	
Maternal working status (yes)	51 (36.4)	533 (41.7)	505 (40.9)	407 (31.7)	≤ .001
Maternal age					≤ .001
≤ 24	66 (47.1)	524 (41.0)	425 (34.4)	485 (37.8)	
25-29	21 (15.0)	294 (23.0)	311 (25.2)	258 (20.1)	
30-34	27 (19.3)	239 (18.7)	284 (23.0)	265 (20.7)	
≥ 35	26 (18.6)	220 (17.2)	214 (17.3)	274 (21.4)	
Maternal IQ					
WAIS digit (below average)	103 (73.6)	825 (64.6)	768 (62.2)	918 (71.6)	≤ .001
WAIS vocabulary (below average)	73 (52.1)	534 (41.8)	502 (40.7)	671 (52.3)	≤ .001
Maternal depression during pregnancy (yes)	23 (16.4)	178 (13.9)	185 (15.0)	188 (14.7)	*ns*
Smoking during pregnancy (yes)	19 (13.6)	141 (11.0)	101 (8.2)	151 (11.8)	.011
Alcohol use during pregnancy (yes)	14 (10.0)	96 (7.5)	77 (6.2)	96 (7.5)	*ns*
Drugs use during pregnancy (yes)	4 (2.9)	7 (0.5)	8 (0.6)	10 (0.8)	.025
Delivery mode (caesarean)	72 (51.4)	606 (47.5)	578 (46.8)	503 (39.2)	≤ .001
Birth weight (≥2500g, yes)	11 (7.9)	34 (2.7)	30 (2.4)	28 (2.2)	.001
Stay in incubator (yes)	12 (8.6)	46 (3.6)	44 (3.6)	49 (3.8)	.030
Infant sex (boy)	67 (47.9)	658 (51.5)	637 (51.6)	630 (49.1)	*ns*

Note: The health provisional system was used as a proxy for the financial status of the family, whereby families in the private system are generally of higher income. Maternal IQ was assessed using both the digit and vocabulary scales of the Wechsler Intelligence Adult Scale, WAIS (Wechsler, 1939). A below average score is defined as a score of 8 or below on the digit and vocabulary scales.

Table 2: *Bivariate Correlations and Means (Standard Deviations) of Children's Behaviours*

	Emotionally reactive	Anxious/ depressed	Somatic complaints	Withdrawn	Sleep problems	Attention problems	Aggressive
Emotionally reactive							
Anxious/depressed	.650**						
Somatic complaints	.549**	.499**					
Withdrawn	.569**	.548**	.435**				
Sleep problems	.445**	.404**	.373**	.350**			
Attention problems	.428**	.374**	.319**	.345**	.341**		
Aggressive	.664**	.542**	.409**	.496**	.477**	.619**	
Mean	3.11	4.33	3.10	2.92	2.83	3.72	13.68
Standard deviation	2.94	3.01	2.63	2.38	2.62	1.97	8.35

Note: ** denotes statistical significance at the 0.01 level

Table 3: *Breastfeeding and Children's Behavioural Problems: Pre and Post Matching Results*

Up to 6 months	Pre Matching				Post Matching			
	T	C	Diff (Sig.)	S.E	T	C	Diff (Sig.)	S.E
Emotionally reactive	3.16	3.98	**-0.81****	0.30	3.16	4.17	**-1.00***	0.43
Anxious/depressed	4.38	4.67	-0.29	0.29	4.38	4.59	-0.21	0.39
Somatic complaints	3.09	3.73	**-0.64***	0.25	3.09	4.11	**-1.02****	0.38
Withdrawn	3.00	3.16	-0.15	0.24	3.00	3.15	-0.15	0.31
Sleep problems	2.86	3.07	-0.20	0.26	2.86	3.40	-0.53	0.36
Attention problems	3.79	4.05	-0.26	0.19	3.79	3.85	-0.06	0.22
Aggression	13.91	14.90	-0.98	0.85	13.91	14.65	-0.74	1.18
Between 7 and 12 months								
Emotionally reactive	2.85	3.98	**-1.12*****	0.29	2.85	3.71	**-0.86***	0.41
Anxious/depressed	4.12	4.67	-0.54	0.30	4.12	4.39	-0.26	0.39
Somatic complaints	2.95	3.73	**-0.78****	0.26	2.95	3.65	-0.70	0.37
Withdrawn	2.70	3.16	**-0.45***	0.22	2.70	3.06	-0.36	0.30
Sleep problems	2.68	3.07	-0.38	0.26	2.68	2.93	-0.25	0.35
Attention problems	3.57	4.05	**-0.48***	0.19	3.57	4.07	**-0.50***	0.22
Aggression	13.02	14.90	**-1.87***	0.81	13.02	13.85	-0.83	1.16
13 months or more								
Emotionally reactive	3.20	3.98	**-0.77***	0.30	3.20	3.66	-0.45	0.48
Anxious/depressed	4.42	4.67	-0.24	0.31	4.42	4.53	-0.10	0.43
Somatic complaints	3.18	3.73	**-0.55***	0.27	3.18	3.43	-0.25	0.42
Withdrawn	3.01	3.16	-0.15	0.24	3.01	3.17	-0.16	0.34
Sleep problems	2.92	3.07	-0.15	0.27	2.92	2.65	0.26	0.40
Attention problems	3.76	4.05	-0.29	0.20	3.76	3.77	-0.01	0.24
Aggression	13.91	14.90	-0.98	0.86	13.91	12.44	1.46	1.32

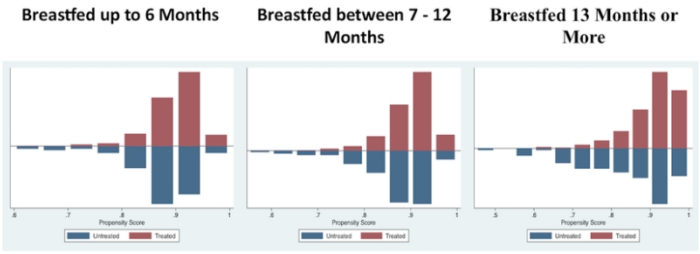
Note: *** denotes significance at the p = < .001 level, ** at the .01 level, * at the .05 level. T denotes 'treatment' (breastfed) and C denotes 'control' (not breastfed). 'Diff' represents the difference in scores between groups. S.E. refers to the standard errors. For being breastfed up to 6 months: N for the treatment group was 949 and 110 for the control group. For being breastfed between 7 and 12 months: N for the treatment group was 946 and 110 for the control group. For being breastfed 13 months or more: N for the treatment group was 1,006 and 110 for the control group.

Figure 1: Overlapping Support: Distribution of Propensity Scores

Note: Treated refers to children who were breastfed, untreated refers to children who were not. For being breastfed up to 6 months: N for the treatment group was 949 and 110 for the control group. For being

breastfed between 7 and 12 months: N for the treatment group was 946 and 110 for the control group. For being breastfed 13 months or more: N for the treatment group was 1,006 and 110 for the control group.

Figure 2: Standardised Differences across Covariates: Pre and Post Matching
Note: Treated refers to children who were breastfed, untreated refers to children who were not. For being breastfed up to 6 months: N for the treatment group was 949 and 110 for the control group. For being breastfed between 7 and 12 months: N for the treatment group was 946 and 110 for the control group. For being breastfed 13 months or more: N for the treatment group was 1,006 and 110 for the control group.



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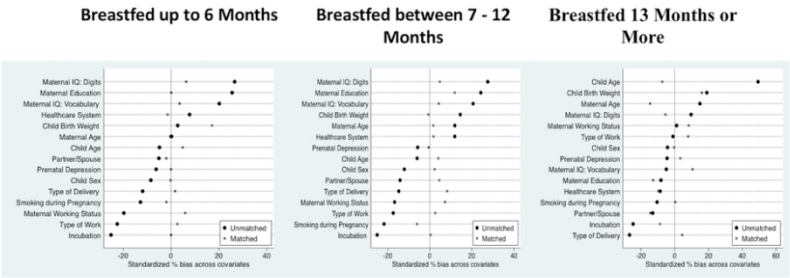

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Online Supplement 1

Family, maternal, infant, and medical characteristics: A comparison between the Entire and Included Cohort

	Entire Cohort N = 14,161 (%)	**Included Families N = 3,933 (%)**	***p***
Resident spouse/partner (yes)	6,873 (69.2%)	2,691 (68.4%)	*ns*
Social class			≤ .001
Professional/managerial	968 (9.7%)	333 (8.5%)	
Non-manual/skilled manual	3,812 (38.4%)	1,404 (35.7%)	
Semi-skilled/unskilled	374 (3.8%)	95 (2.4%)	
Never worked	4,779 (48.1%)	2,101 (53.4%)	
Health provisional system			*ns*
Public system	8,820 (88.8%)	3,508 (89.2%)	
Private system	1,113 (11.2%)	425 (10.8%)	
Maternal education			*ns*
No formal education	37 (0.4%)	14 (0.4%)	
Primary complete	1,743 (17.7%)	614 (15.7%)	
Secondary Complete	3,917 (39.8%)	1,577 (40.4%)	
Vocational training	2,984 (30.3%)	1,222 (31.3%)	
University training	1,075 (10.9%)	438 (11.2%)	
Postgraduate studies	81 (0.8%)	37 (0.9%)	
Maternal working status (yes)	4,366 (44.0%)	1,496 (38.0%)	≤ .001
Maternal age			≤ .001
≤ 24	2,806 (28.2%)	1,500 (38.1%)	
25-29	2,250 (22.7%)	884 (22.5%)	
30-34	2,173 (21.9%)	815 (20.7%)	
≥ 35	2,704 (27.2%)	734 (18.7%)	
Maternal depression during pregnancy (yes)	1,027 (10.3%)	574 (14.6%)	≤ .001
Smoking during pregnancy (yes)	978 (9.9%)	412 (10.5%)	*ns*
Alcohol use during pregnancy (yes)	705 (7.1%)	283 (7.2%)	*ns*
Drug use during pregnancy (yes)	81 (0.8%)	29 (0.7%)	*ns*
Delivery mode (caesarean)	4,314 (43.5%)	1,759 (44.7%)	*ns*
Birth weight (≥ 2500g, yes)	205 (2.3%)	103 (2.6%)	*ns*
Stay in incubator (yes)	433 (4.4%)	151 (3.8%)	*ns*
Infant sex (boy)	5,004 (50.4%)	1,992 (50.6%)	*ns*

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract: In both, Pgs. 1 and 2.
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Pg. 2.
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Pgs. 3-4
Objectives	3	State specific objectives, including any prespecified hypotheses. Pg. 4
Methods		
Study design	4	Present key elements of study design early in the paper Pgs. 4-5.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Pgs. 4-5.
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Pg. 5 *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed Pgs. 5-6. + Table 3 and Figures 1 and 2. *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Pgs. 5-7 + Figure 2.
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Pgs. 5-6.
Bias	9	Describe any efforts to address potential sources of bias Pgs. 6-7 + Figure 2
Study size	10	Explain how the study size was arrived at Pgs. 4-5.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Pgs. 5-6.
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding Pgs. 6-7
		(*b*) Describe any methods used to examine subgroups and interactions N/A
		(*c*) Explain how missing data were addressed Pg. 9 and Online Supplement
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses N/A

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Table 1 and 3
		(b) Give reasons for non-participation at each stage Pg. 4
		(c) Consider use of a flow diagram N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Table 1 and 2
		(b) Indicate number of participants with missing data for each variable of interest N/A
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) Pg. 4
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time Tables 2 and 3
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Abstract (pg. 2), Table 3 and Figure 2
		(*b*) Report category boundaries when continuous variables were categorized Pg. 6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses N/A
Discussion		
Key results	18	Summarise key results with reference to study objectives Pgs. 7-8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Pgs. 8-9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Pgs. 7-9
Generalisability	21	Discuss the generalisability (external validity) of the study results Pg. 9
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Breastfeeding and behavioural problems: Propensity score matching with a national cohort of infants in Chile

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-025058.R2
Article Type:	Research
Date Submitted by the Author:	14-Dec-2018
Complete List of Authors:	Lisa-Christine , Girard; University of Edinburgh , Clinical Psychology Farkas, Chamarrita; Pontificia Universidad Catolica de Chile, Psychology
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Paediatrics, Nutrition and metabolism, Public health
Keywords:	Breastfeeding, Behaviour Problems, Cohort Study

Breastfeeding and behavioural problems: Propensity score matching with a national cohort of infants in Chile

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Short title: Breastfeeding and behaviour

Abbreviations: Encuesta Longitudinal de la Primera Infancia cohort, ELPI; Propensity Score Matching PSM; Child Behaviour Checklist CBCL; World Health Organization WHO; Randomized Control Trial RCT; Attention to Treat ATT

Author Contributions:
Dr. Girard and Prof. Farkas conceptualized the study, carried out the analyses, interpreted the data, drafted the initial manuscript, critically reviewed and revised the manuscript, and approved the final manuscript as submitted. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Data Access, Responsibility, and Analysis: Dr. Girard and Prof. Farkas had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Data Sharing Statement:
Data is not able to be deposited in an open repository however researchers wishing to access the data may do so directly by writing a request to the Centro de Microdatos de la Facultad de Economía y Negocios de la Universidad de Chile.

Conflict of Interest Statement: The authors have no conflicts of interest relevant to this article to disclose.

Financial Disclosure Statement: The authors have no financial relationships relevant to this article to disclose.

Word Count: 3000

Key words: Breastfeeding; Behaviour Problems; Propensity Score Matching; Epidemiology; Developmental Cohort

Abstract

Importance: Potential effects of breastfeeding on children's behaviour remains an elusive debate given inherent methodological challenges. Propensity score matching affords benefits by ensuring greater equivalence on observable social and health determinants, helping to reduce bias between groups.

Objectives: We examined whether duration of breastfeeding had an impact on children's externalising and internalising behaviours.

Study Design: A cohort study (Encuesta Longitudinal de la Primera Infancia cohort) that included 3,037 Chilean families who were enrolled in 2010. Follow-up data was collected in 2012.

Setting: General community.

Participants: Population-based sample. Eligibility criteria: children born full-term with complete data on matching variables. Matching variables included: healthcare system as a proxy of income, presence of a partner/spouse in the household, maternal age, educational level, IQ, working status, type of work, diagnosis of prenatal depression by a healthcare professional, smoking during pregnancy, delivery type, child sex, weight at birth, incubation following delivery, and child age.

Exposure: Duration of breastfeeding.

Main Outcomes and Measures: Externalising and internalising problems assessed using the Child Behaviour Checklist.

Results: Matched results revealed benefits of any breastfeeding, up to six months, on emotional reactivity and somatic complaints, (mean difference of -1.00, 95% CI, -1.84 to -0.16 and -1.02, 95% CI, -1.76 to -0.28, respectively). Children breastfed between 7-12 months also had reduced scores on emotional reactivity, in addition to attention problems (mean difference of -0.86, 95% CI, -1.66 to -0.06 and -0.50, 95% CI, -0.93 to -0.07, respectively). No benefits were observed for children breastfed 13 months or more.

Conclusion: Reduced internalising difficulties and inattention were found in children breastfed up to a year, suggesting that breastfeeding may have beneficial impacts on these areas of development. The magnitude of effect was modest. Extended durations of breastfeeding did not appear to offer any benefits.

Article Summary:

Strengths:

- Use of a quasi-experimental statistical approach to match children with the propensity to be breastfed to those who were not on observable health and social determinates.
- Use of a large Chilean cohort where confounding structure differs from developed countries.
- The inclusion of 14 matching variables including maternal IQ, which is almost double the average amount of variables included in similar studies.

Limitations:

- No specific information was collected on full breastfeeding in this cohort restricting the study to examining duration only.
- As a result of the inclusion/exclusion criteria, the sample size was reduced from the entire cohort.

Introduction

A considerable amount of literature supports numerous medical benefits of breastfeeding for children in reducing for example, the risk of nonspecific gastroenteritis, severe lower respiratory tract infections, atopic dermatitis, obesity, and high systolic blood pressure.[1-3] However, the benefits of breastfeeding on children's behavioural outcomes are less clear-cut. Differing hypotheses have been put forth regarding potential mechanisms for the perceived associations between breastfeeding and behavioural outcomes. For example, it has been suggested that breastfeeding may lead to reduced behavioural difficulties as a result of early skin-to-skin contact, when active bonding is present, helping to promote the development of a secure bond between mother and baby.[e.g.,4] This may be particularly salient in protecting against the emergence of internalising behaviours as children develop.[5] On the other hand, associations between breastfeeding and behavioural outcomes may be the result of the long-chain polyunsaturated fatty acids (n-3 and n-6 PUFA) found in breast milk, which arguably impact on brain development and white growth matter; an area of the brain which is typically underdeveloped in children who display with elevated levels of externalising behaviours.[6-7] Moreover, deficits in n-3 PUFA in particular, have been shown to increase the risk of neuronal abnormalities in studies of rats, associated with increased levels of anxiety, depression, aggression, inattention and hyperactivity.[e.g.,8-10] While the mechanisms suggested have differing implications regarding pathways of potential effects, it is important to first be able to untangle whether 'effects' exist for children's behavioural outcomes, irrespective of selection and confounding. For example, it may be possible that there is no direct mechanism through which breastfeeding is implicated in behavioural outcomes, but rather, associations found may be an artefact of maternal and family-level characteristics.

There has been mixed support for associations between breastfeeding and externalising behaviours such as conduct problems and hyperactivity[e.g.,11-16], and internalising behaviours such as anxiety and depression[e.g., 5,17-19], in studies from infancy to adulthood. When associations are observed, a duration of four-to-six months or longer appears to be most common. Systematic differences between studies regarding definitions of breastfeeding, classification of behaviours, timing of assessment, and statistical approaches for handling confounding and selection bias, are likely contributing factors accounting for these inconsistencies. For example, self-selection into breastfeeding has repeatedly been implicated in studies examining breastfeeding and developmental outcomes. In developed countries in particular, research continues to demonstrate characteristics common in mothers who breastfeed (e.g., lower engagement in high-risk prenatal behaviours, higher education, higher income, and older age at child birth[e.g.,20-21]), which are also associated with behavioural outcomes. If associations between breastfeeding and behavioural difficulties are an artefact of maternal or family characteristics, differences in statistical approaches for handling selection bias will have important consequences. Indeed, this is reflected in the literature whereby the greater the number of implicated confounders are controlled, often, the less likely significant associations remain.[22]

On the other hand, using cohorts from developing countries may provide additional insights given the differing confounding structures.[23] For example, notable differences between developed and developing countries regarding associated maternal characteristics were recently demonstrated in the Lancet series.[21] More specifically, maternal characteristics common of mothers in developing countries who breastfeed, particularly for longer durations, included poverty, lower socio-economic-status, and in some cases, lower maternal education.[24-25] Given this inverse association between socio-economic standing and selection into breastfeeding, replication of associations between breastfeeding and behavioural problems, may offer additional advantages in better understanding potential 'effects'. Currently, there are a lack of studies examining breastfeeding and behavioural outcomes using nationally representative cohorts of infants in Chile, which provides such a possibility. While economic growth has been observed, social inequalities in Chile remain high, particularly for women[26]. Additionally, challenges in examining associations between longer durations of breastfeeding on behavioural outcomes due to low prevalence rates in many developed countries is common. In Chile, duration of breastfeeding has been steadily increasing over the past decade and was reported in 2014 at 63% of mothers who were still exclusively breastfeeding when infants were six-months of age.[27] Taken together, examining breastfeeding duration and behavioural outcomes with a nationally representative cohort from Chile, may offer additional benefits in our understanding.

Objectives

To examine breastfeeding and children's behavioural outcomes longitudinally, using a quasi-experimental statistical technique to reduce observable differences between groups, whereby attempting to address inherent limitations in observational studies. Duration of breastfeeding was examined. Moreover, we examined whether in using a Chilean cohort, we could replicate the findings of Girard et al.[11-12] regarding reduced hyperactivity for children breastfed, following propensity score matching, in two separate longitudinal Irish cohorts. We extend upon this work by also examining internalising behaviours. We hypothesised, in line with previous findings and recommendations of the WHO[28], that children who were breastfed for a minimum of six-months would present with reduced behavioural problems in early childhood.

Method

Participants included families enrolled in the "Encuesta Longitudinal de la Primera Infancia cohort, ELPI", recruited in Chile in 2010 and 2012. Families recruited in the second wave (i.e., in 2012) were not considered in this study given that child outcomes were not available longitudinally. The cohort was initially recruited to better understand the sociodemographic backgrounds of children and their families alongside their physical, social, and emotional development over time. The cohort is representative of children born between January 2006 and August 2009 in urban and rural areas, across all regions of Chile.[29] A total of

15,175 families with children between the ages of seven and 58-months were initially contacted for inclusion. At wave one, 14,161 families were assessed, 93.3% of the targeted sample. However, 487 children did not have information pertaining to their age and were excluded. Inclusion criteria in this study were children aged seven to 24-months, who had complete data on all confounders at wave one, and who were born full term (n=4,375). Additionally, mothers who were still breastfeeding at wave one who had breastfed more than six, but less than 12-months, were excluded (n=442), as it was not possible to identify whether they should be included in the group of children breastfed between seven and 12-months or in the extended breastfeeding group. This resulted in a possible sample of 3,933 children and their families, 50.6% of whom were boys (n=1,992) at wave one. However, missing outcome data (i.e., child behaviours) at wave two in 2012, resulted in a final sample 3,037. Demographic characteristics of the included children and their families can be found in Table 1. A table comparing the entire cohort to those included can be found in the online supplement. Ethical approval was obtained from the Universidad de Chile, Centro de Microdatos. Parents/guardians were provided with oral and written information about the study and informed consent was given with completion of the first questionnaire.

Children's behaviours were assessed using the Child Behaviour Checklist 1½-5 (CBCL)[30]. The CBCL 1½-5 is a parent report used to identify behaviour problems in children between the ages of one-and-a-half and five years. It is comprised of 99 items divided into subscales. The seven subscales include: emotionally reactive (9 items), anxious/depressed (8 items), somatic complaints (11 items), withdrawn (8 items), sleep problems (7 items), attention problems (5 items), and aggression (19 items). Parents rate each individual behavioural item on 3-point Likert scale ranging from 0 (*not true*) to 2 (*very true or often true*), with higher scores indicative of more problematic behaviour. The CBCL 1½-5 is available in several languages, including Spanish, and has previously been validated in the literature with Chilean samples.[(e.g., 31)] The CBCL was collected at wave two, in 2012, when children were between the ages of 32 and 48-months. Means and standard deviations, along with correlations between subscales are presented in Table 2.

Breastfeeding information was collected at wave one, both retrospectively and prospectively, given the unique sampling approach of recruitment of families with children between the ages of seven and 58 months. Due to potential recall bias and in an attempt to create more homogeneity within mother's duration of recall, we included only families with children between seven and 24-months. Mothers were asked two questions: "was the newborn breastfed by his/her biological mother?" and "until what month was the child breastfed by his/her biological mother?" No information was collected regarding full breastfeeding in the cohort. Of the sample included, only 140 mothers/caregivers reported that the child had never been breastfed. Based on the WHO recommendations, we grouped children into one of four categories of duration; never breastfed (*n*=140), breastfed up to six complete months (*n* = 1,277), breastfed between seven and 12

complete months (n=1,234), and breastfed ≥ 13 months (n = 1,282). Each category of duration was treated as mutually exclusive, dummy coded, and compared against children who had never been breastfed.

Numerous confounders and self-selection into breastfeeding have been argued to account, at least in part, for previous associations between breastfeeding and behavioural outcomes. In the current study we matched groups on 13 of the most commonly identified factors, along with children's age given the variation in this sample. At the family level these included the category of the healthcare system that the family belonged to (public, private) as a proxy of income, and the presence of a partner/spouse in the household (yes/no). To note, the quality of services offered in the private and public healthcare system in Chile differ vastly, with higher quality services offered in the private system; subsequently translating into a high cost of belonging to the private system. Moreover, for those employed, the tier of the healthcare system in which one belongs is directly related to salary, whereby employers pay into the healthcare system on their employees behalf, which is a calculated monthly percentage deductable, based on individual income earnings. At the maternal level, confounders included maternal age, educational level (no formal education, primary, secondary, vocational training, university training, postgraduate), maternal IQ (a score of 8 or below on the Wechsler Intelligence Adult Scale, digit and vocabulary scales; WAIS,[32]), working status (yes/no), type of work (professional/managerial, non-manual/skilled manual, semi-skilled/unskilled, unknown/never worked), diagnosis of depression by a healthcare professional during pregnancy (yes/no), smoking during pregnancy (yes/no), and delivery type (vaginal, caesarean). To note, the WAIS has been adapted in Chile with good reported reliability and validity.[33-34] At the child level, four confounders were included, namely, child sex (boy/girl), weight at birth (≥2500 grams, yes/no), whether the child was placed in an incubator after delivery (yes/no), and age at first assessment in wave 1.

Patient and Public Involvement

The development of the research question and outcome measures, along with study design and recruitment to, were not directly informed by patients' priorities, experience or preference. Study findings will be disseminated to the Ministry of Labor and Social Welfare, whom were responsible for waves 1 and 2, and the Ministry of Social Development, who is currently responsible for wave 3 of the ELPI cohort, ensuring greater likelihood of dissemination to study participants.

Statistical Analysis

We employed the use of propensity score matching (PSM), a statistical approach which attempts to ensure equivalence between treatment and control groups (i.e., breastfed, not breastfed), by matching groups on the most relevant factors, subsequently reducing selection bias and confounding. That is, comparisons are made between children who were breastfed and those who were not based on their measured characteristics and similar propensities for being breastfed. Nearest neighbour 1:1 models, with replacement were used. In nearest neighbour matching, groups are first randomly ordered to reduce possible bias in the matching

procedure, with matching then occurring sequentially. To ensure the most optimal matches between pairs on propensity scores, we imposed a caliper of a tenth of a standard deviation. That is, for a match to occur, the propensity score of a child who was breastfed to a child who was not, had to fall within a tenth of a standard deviation of one another. Matching with replacement was necessary given the low rates of children who were never breastfed. While this technique can result in larger amounts of variance, it has been argued to reduce bias by ensuring matches are of better quality.[35] All children fell within the area of common support which refers to cases being excluded as a result of not fitting within the specified caliper. See Figure 1 for the overlapping support of the distribution of propensity scores. To ensure the overall quality of the matching procedure, balance checks were conducted on individual confounders and the overall models. For individual factors, remaining bias ranged between 0.0 and 18.8% (see Figure 2) and the overall mean remaining bias for models ranged between 5.5% and 8.3%. It has been suggested that less than 20% remaining bias is indicative of good matching,[36] thus we concluded that our matching was successful. We report on the average treatment effects of those treated (ATT). All analyses were conducted using Stata v14 software. We use the term significant henceforth to denote statistical significance, using a threshold of $p = <.05$.

Results

Comparing children never breastfed to those who were breastfed up to six months inclusive, significant differences in favour of those who were breastfed were found on two of the behavioural subscales (i.e., emotionally reactive and somatic complaints). These results remained significant following matching whereby children who were breastfed had lower scores on these subscales (i.e., a mean difference of -1.00, d = -0.23 and -1.02, d = -0.27 respectively). Comparing children who were never breastfed to those breastfed between seven and 12 months inclusive, significant differences were found prior to matching on all subscales with the exception of anxious/depressed and sleep problems. After matching, significant differences remained for emotional reactivity and attention problems only (i.e., a mean difference of -0.86, d = -0.21 and -0.50, d = -0.22, respectively), with reduced difficulties for children who were breastfed. Comparing those never breastfed to the extended group (i.e., 13 months or more), pre-matching results were similar to the first model with lower scores on the emotionally reactive and somatic complaints subscales. Post matching revealed no remaining significant differences between groups. All results can be found in Table 3.

Discussion

While causality remains an ongoing debate, owing to the ethical constraints of conducting RCTs, our results add to the emerging corpus of literature trying to untangle the 'effects' of breastfeeding on behavioural outcomes by using a PSM approach in a Chilean cohort. Given the knowledge of family and maternal characteristics commonly observed of children who are breastfed, the use of matching helps to ensure that differences between groups on these observable characteristics are significantly minimised, so

that the propensity to be breastfed across groups is arguably comparable. As a result, only post-matching results are discussed. Due to exclusions, our results have implications only for children who were born full term.

The results suggest that breastfeeding may be an effective low-cost early investment for reducing difficulties with emotional reactivity and somatic complaints, in addition to attention problems experienced in early childhood; all of which are indicative of neurodevelopmental difficulties. Duration appears to be an important factor implicated in these associations. For example, results revealed that any breastfeeding during the first six months, and up to 12 full months, contributed to reductions in children's difficulties with emotional reactivity and somatic complaints up to four years of age. In comparison, a minimum of at least six full months or more of breastfeeding was required for observed benefits related to attention problems at the same age. This latter finding is in line with findings from Girard et al.,[11], who also used PSM in a nationally representative sample of infants from Ireland. The similar results found around three years of age, across these two unique cohorts, whilst using PSM, demonstrates favourable support for a direct mechanism between breastfeeding and reduced attention problems in early childhood, rather than confounding per se. Worth noting is the link found between inattention/hyperactivity, regulation difficulties and mood disorders[37], and their link with deficiencies in arachidonic acid and docosahexaenoic acid.[37] Given the types of behaviours where reductions for those breastfed are found in this study, and in the context of previous studies [(e.g.,8-10, 37-38)], a plausible hypothesis might be that of the nutrients found in breastmilk contributing to the growing infant's brain development. More research in this area, using well-designed and rigorously sound methodology is first needed before firm conclusions can be drawn.

While our results suggest statistically significant differences in favour of children who were breastfed at least six full months (and up until 12 full months), as compared to those who were never breastfed on emotional reactivity, somatic complaints, and inattention, the magnitude of effect for each behaviour was found to be small (i.e., Cohen's $d = < .30$). The practical and clinical significance of our results is arguably interpretable in the eye of the 'stakeholder'. A small reduction in a child's emotional reactivity, somatic complaints, and/or inattention in everyday situations may carry greater importance to a first-time or multiparous mother experiencing high levels of stress and fatigue as a result of limited financial and/or personal resources. On the other hand, within a clinical context, the effect sizes found may be perceived as carrying less practical importance.

Conversely, no benefits of extended breastfeeding were found on children's internalising or externalising behavioural problems. This may suggest a non-linear dose-response effect of breastfeeding on behaviour, similar to previous findings. [(e.g.,12)] While the recommendations put forth by the WHO for continued partial breastfeeding up to two years of age or more for the physical health and growth of infants has been suggested, we observed no additional benefits of extended breastfeeding on behavioural problems in

this sample. These findings do not however contradict these recommendations regarding the many afforded medical benefits of extended durations of breastfeeding. Of interest and as can be seen in Table 1, mothers who breastfed for extended durations in Chile had similar characteristics to mothers who had never breastfed, lending to poorer quality matching. For example, in both the never- and extended breastfeeding groups, a significantly higher proportion of mothers had never worked, were in the public tier of the health system, had only completed education at the primary level and had below average scores on both the digit and vocabulary scales of the WAIS; factors which when previously controlled, have reduced observed associations between breastfeeding and children's cognitive and behavioural development outcomes. While a non-linear dose-response hypothesis is plausible, these findings also likely support what is already known. Maternal characteristics contribute to children's behavioural outcomes and the nutrients alone found in breastmilk may be only part of the story. Moreover, it supports the differences in confounding structures of breastfeeding in developing and developed countries. [21,23]

Notable strengths of this study include the use of the largest nationally representative cohort of Chilean children to date, where self-selection and confounding structures differ from developed countries, whilst utilising PSM, with a large number of matching confounders to reduce differences across groups, of which included maternal IQ. Despite these strengths, notable limitations must be mentioned. While information on necessary supplementation was asked, there was no specific information regarding full breastfeeding, limiting our ability to examine its impact. This is an important issue given the differences in feeding experiences and the potential for dilution of effects from breastfeeding to behaviour. In the same vein, no information regarding direct breastfeeding versus expressed breast milk was collected; information which may help in better understanding pathways of effect. Due to our inclusion/exclusion criteria, the sample size was significantly reduced, with some statistically significant differences between the originally recruited cohort and those included in the current study, indicative of potential selection bias. Thus, warranting replication. Additionally, despite the benefits afforded by PSM techniques, matching is only possible on observable characteristics. While we were able to include a multitude of health and social confounders, including maternal IQ, it remains possible that unobservable characteristics contribute to the associations. Relatedly, the quality of matching for the extended breastfeeding families as compared to the never breastfeeding families was not as successful compared to the matching between the other groups, due to the initial similarities on health and social factors. The included covariates used for matching were theoretically motivated and thus, we kept the integrity of matching variables intact across all models. However, the findings from this model (i.e., the extended breastfeeding families) warrants caution in interpretation. Future studies are needed to more carefully evaluate extended breastfeeding and potential associations with behavioural outcomes, in the context of differing confounding structure. Finally, shared

method variance is a concern given that parent reports were used to collect information on both breastfeeding duration and child behaviours.

Despite these limitations, and in the context of the strengths of this study, we believe these results contribute important findings, namely more support for the potential of 'causal paths'. A comprehensive answer to the question of effects on psychosocial development remains unanswered without the use of RCTs. However, with replication across regions, whilst using more stringent methodological approaches to help in reducing bias inherent in observational studies, promise for better understanding of potential mechanisms is viable.

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Table 1: *Family, maternal, infant, and medical characteristics: Infant cohort between 7 and 24 months*

	Never breastfeed (n=140)	1-6 months (n=1277)	7-12 months (n=1234)	13 months or more (n=1282)	*p*
	n (%)	**n (%)**	**n (%)**	**n (%)**	
Resident spouse/partner (yes)	90 (64.3)	851 (66.6)	859 (69.6)	891 (69.5)	*ns*
Social class					≤ .001
Professional/managerial	8 (5.7)	111 (8.7)	98 (7.9)	116 (9.0)	
Non-manual/skilled manual	50 (35.7)	512 (40.1)	487 (39.5)	355 (27.7)	
Semiskilled/unskilled	5 (3.6)	33 (2.6)	23 (1.9)	34 (2.7)	
Never worked	77 (55.0)	621 (48.6)	626 (50.7)	777 (60.6)	
Health provisional system					≤ .001
Public system	127 (90.7)	1107 (86.7)	1069 (86.6)	1205 (94.0)	
Private system	13 (9.3)	170 (13.3)	165 (13.4)	77 (6.0)	
Maternal education					≤ .001
No formal education	0 (0)	3 (0.2)	4 (0.3)	7 (0.5)	
Primary complete	26 (18.6)	155 (12.1)	154 (12.5)	279 (21.8)	
Secondary complete	59 (42.1)	491 (38.4)	483 (39.1)	544 (42.4)	
Vocational training	42 (30.0)	441 (34.5)	392 (31.8)	347 (27.1)	
University training	13 (9.3)	163 (12.8)	176 (14.3)	86 (6.7)	
Postgraduates studies	0 (0)	13 (1.0)	17 (1.4)	7 (0.5)	
Unknown	0 (0)	11 (0.9)	8 (0.6)	12 (0.9)	
Maternal working status (yes)	51 (36.4)	533 (41.7)	505 (40.9)	407 (31.7)	≤ .001
Maternal age					≤ .001
≤ 24	66 (47.1)	524 (41.0)	425 (34.4)	485 (37.8)	
25-29	21 (15.0)	294 (23.0)	311 (25.2)	258 (20.1)	
30-34	27 (19.3)	239 (18.7)	284 (23.0)	265 (20.7)	
≥ 35	26 (18.6)	220 (17.2)	214 (17.3)	274 (21.4)	
Maternal IQ					
WAIS digit (below average)	103 (73.6)	825 (64.6)	768 (62.2)	918 (71.6)	≤ .001
WAIS vocabulary (below average)	73 (52.1)	534 (41.8)	502 (40.7)	671 (52.3)	≤ .001
Maternal depression during pregnancy (yes)	23 (16.4)	178 (13.9)	185 (15.0)	188 (14.7)	*ns*
Smoking during pregnancy (yes)	19 (13.6)	141 (11.0)	101 (8.2)	151 (11.8)	.011
Alcohol use during pregnancy (yes)	14 (10.0)	96 (7.5)	77 (6.2)	96 (7.5)	*ns*
Drugs use during pregnancy (yes)	4 (2.9)	7 (0.5)	8 (0.6)	10 (0.8)	.025
Delivery mode (caesarean)	72 (51.4)	606 (47.5)	578 (46.8)	503 (39.2)	≤ .001
Birth weight (≥2500g, yes)	11 (7.9)	34 (2.7)	30 (2.4)	28 (2.2)	.001
Stay in incubator (yes)	12 (8.6)	46 (3.6)	44 (3.6)	49 (3.8)	.030
Infant sex (boy)	67 (47.9)	658 (51.5)	637 (51.6)	630 (49.1)	*ns*

Note: The health provisional system was used as a proxy for the financial status of the family, whereby families in the private system are generally of higher income. Maternal IQ was assessed using both the digit and vocabulary scales of the Wechsler Intelligence Adult Scale, WAIS (Wechsler, 1939). A below average score is defined as a score of 8 or below on the digit and vocabulary scales.

Table 2: *Bivariate Correlations and Means (Standard Deviations) of Children's Behaviours*

	Emotionally reactive	Anxious/ depressed	Somatic complaints	Withdrawn	Sleep problems	Attention problems	Aggressive
Emotionally reactive							
Anxious/depressed	.650**						
Somatic complaints	.549**	.499**					
Withdrawn	.569**	.548**	.435**				
Sleep problems	.445**	.404**	.373**	.350**			
Attention problems	.428**	.374**	.319**	.345**	.341**		
Aggressive	.664**	.542**	.409**	.496**	.477**	.619**	
Mean	3.11	4.33	3.10	2.92	2.83	3.72	13.68
Standard deviation	2.94	3.01	2.63	2.38	2.62	1.97	8.35
Min-Max	0 - 18	0 - 16	0 - 22	0 - 16	0 - 14	0 - 10	0 - 38

Note: ** denotes statistical significance at the 0.01 level.

Table 3: *Breastfeeding and Children's Behavioural Problems: Pre and Post Matching Results*

Up to 6 months	Pre Matching				Post Matching			
	T	C	Diff (Sig.)	S.E	T	C	Diff (Sig.)	S.E
Emotionally reactive	3.16	3.98	**-0.81****	0.30	3.16	4.17	**-1.00***	0.43
Anxious/depressed	4.38	4.67	-0.29	0.29	4.38	4.59	-0.21	0.39
Somatic complaints	3.09	3.73	**-0.64***	0.25	3.09	4.11	**-1.02****	0.38
Withdrawn	3.00	3.16	-0.15	0.24	3.00	3.15	-0.15	0.31
Sleep problems	2.86	3.07	-0.20	0.26	2.86	3.40	-0.53	0.36
Attention problems	3.79	4.05	-0.26	0.19	3.79	3.85	-0.06	0.22
Aggression	13.91	14.90	-0.98	0.85	13.91	14.65	-0.74	1.18
Between 7 and 12 months								
Emotionally reactive	2.85	3.98	**-1.12*****	0.29	2.85	3.71	**-0.86***	0.41
Anxious/depressed	4.12	4.67	-0.54	0.30	4.12	4.39	-0.26	0.39
Somatic complaints	2.95	3.73	**-0.78****	0.26	2.95	3.65	-0.70	0.37
Withdrawn	2.70	3.16	**-0.45***	0.22	2.70	3.06	-0.36	0.30
Sleep problems	2.68	3.07	-0.38	0.26	2.68	2.93	-0.25	0.35
Attention problems	3.57	4.05	**-0.48***	0.19	3.57	4.07	**-0.50***	0.22
Aggression	13.02	14.90	**-1.87***	0.81	13.02	13.85	-0.83	1.16
13 months or more								
Emotionally reactive	3.20	3.98	**-0.77***	0.30	3.20	3.66	-0.45	0.48
Anxious/depressed	4.42	4.67	-0.24	0.31	4.42	4.53	-0.10	0.43
Somatic complaints	3.18	3.73	**-0.55***	0.27	3.18	3.43	-0.25	0.42
Withdrawn	3.01	3.16	-0.15	0.24	3.01	3.17	-0.16	0.34
Sleep problems	2.92	3.07	-0.15	0.27	2.92	2.65	0.26	0.40
Attention problems	3.76	4.05	-0.29	0.20	3.76	3.77	-0.01	0.24
Aggression	13.91	14.90	-0.98	0.86	13.91	12.44	1.46	1.32

Note: *** denotes significance at the p = < .001 level, ** at the .01 level, * at the .05 level. T denotes 'treatment' (breastfed) and C denotes 'control' (not breastfed). 'Diff' represents the difference in scores between groups. S.E. refers to the standard errors. For being breastfed up to 6 months: N for the treatment group was 949 and 110 for the control group. For being breastfed between 7 and 12 months: N for the treatment group was 946 and 110 for the control group. For being breastfed 13 months or more: N for the treatment group was 1,006 and 110 for the control group.

Figure 1: Overlapping Support: Distribution of Propensity Scores

Note: Treated refers to children who were breastfed, untreated refers to children who were not. For being breastfed up to 6 months: N for the treatment group was 949 and 110 for the control group. For being

breastfed between 7 and 12 months: N for the treatment group was 946 and 110 for the control group. For being breastfed 13 months or more: N for the treatment group was 1,006 and 110 for the control group.

Figure 2: Standardised Differences across Covariates: Pre and Post Matching
Note: Treated refers to children who were breastfed, untreated refers to children who were not. For being breastfed up to 6 months: N for the treatment group was 949 and 110 for the control group. For being breastfed between 7 and 12 months: N for the treatment group was 946 and 110 for the control group. For being breastfed 13 months or more: N for the treatment group was 1,006 and 110 for the control group.

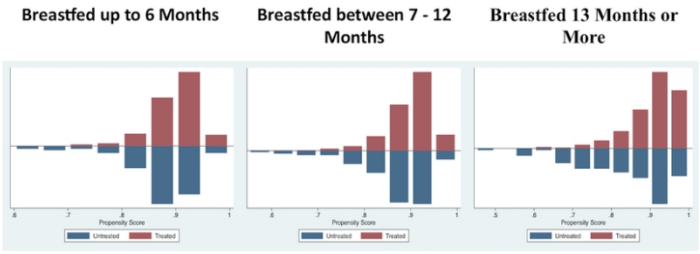

Figure 1: Overlapping Support: Distribution of Propensity Scores
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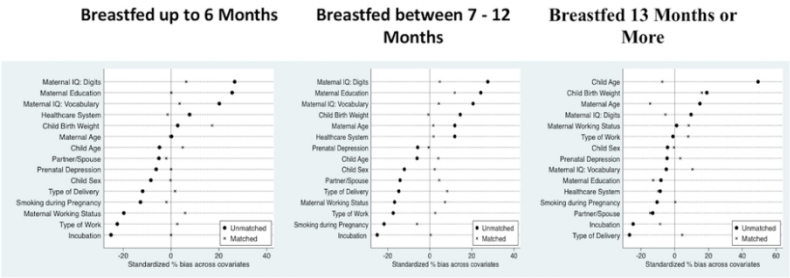

Figure 2: Standardised Differences across Covariates: Pre and Post Matching.
Note: Treated refers to children who were breastfed, untreated refers to children who were not. For being breastfed up to 6 months: N for the treatment group was 949 and 110 for the control group. For being breastfed between 7 and 12 months: N for the treatment group was 946 and 110 for the control group. For being breastfed 13 months or more: N for the treatment group was 1,006 and 110 for the control group.

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Online Supplement 1

Family, maternal, infant, and medical characteristics: A comparison between the Entire and Included Cohort

	Entire Cohort N = 14,161 (%)	**Included Families N = 3,933 (%)**	***p***
Resident spouse/partner (yes)	6,873 (69.2%)	2,691 (68.4%)	*ns*
Social class			≤ .001
Professional/managerial	968 (9.7%)	333 (8.5%)	
Non-manual/skilled manual	3,812 (38.4%)	1,404 (35.7%)	
Semi-skilled/unskilled	374 (3.8%)	95 (2.4%)	
Never worked	4,779 (48.1%)	2,101 (53.4%)	
Health provisional system			*ns*
Public system	8,820 (88.8%)	3,508 (89.2%)	
Private system	1,113 (11.2%)	425 (10.8%)	
Maternal education			*ns*
No formal education	37 (0.4%)	14 (0.4%)	
Primary complete	1,743 (17.7%)	614 (15.7%)	
Secondary Complete	3,917 (39.8%)	1,577 (40.4%)	
Vocational training	2,984 (30.3%)	1,222 (31.3%)	
University training	1,075 (10.9%)	438 (11.2%)	
Postgraduate studies	81 (0.8%)	37 (0.9%)	
Maternal working status (yes)	4,366 (44.0%)	1,496 (38.0%)	≤ .001
Maternal age			≤ .001
≤ 24	2,806 (28.2%)	1,500 (38.1%)	
25-29	2,250 (22.7%)	884 (22.5%)	
30-34	2,173 (21.9%)	815 (20.7%)	
≥ 35	2,704 (27.2%)	734 (18.7%)	
Maternal depression during pregnancy (yes)	1,027 (10.3%)	574 (14.6%)	≤ .001
Smoking during pregnancy (yes)	978 (9.9%)	412 (10.5%)	*ns*
Alcohol use during pregnancy (yes)	705 (7.1%)	283 (7.2%)	*ns*
Drug use during pregnancy (yes)	81 (0.8%)	29 (0.7%)	*ns*
Delivery mode (caesarean)	4,314 (43.5%)	1,759 (44.7%)	*ns*
Birth weight (≥ 2500g, yes)	205 (2.3%)	103 (2.6%)	*ns*
Stay in incubator (yes)	433 (4.4%)	151 (3.8%)	*ns*
Infant sex (boy)	5,004 (50.4%)	1,992 (50.6%)	*ns*

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract: In both, Pgs. 1 and 2.
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Pg. 2.
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Pgs. 3-4
Objectives	3	State specific objectives, including any prespecified hypotheses. Pg. 4
Methods		
Study design	4	Present key elements of study design early in the paper Pgs. 4-5.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Pgs. 4-5.
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Pg. 5 *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed Pgs. 5-6. + Table 3 and Figures 1 and 2. *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Pgs. 5-7 + Figure 2.
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Pgs. 5-6.
Bias	9	Describe any efforts to address potential sources of bias Pgs. 6-7 + Figure 2
Study size	10	Explain how the study size was arrived at Pgs. 4-5.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Pgs. 5-6.
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding Pgs. 6-7
		(*b*) Describe any methods used to examine subgroups and interactions N/A
		(*c*) Explain how missing data were addressed Pg. 9 and Online Supplement
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses N/A

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Table 1 and 3
		(b) Give reasons for non-participation at each stage Pg. 4
		(c) Consider use of a flow diagram N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Table 1 and 2
		(b) Indicate number of participants with missing data for each variable of interest N/A
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) Pg. 4
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time Tables 2 and 3
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Abstract (pg. 2), Table 3 and Figure 2
		(*b*) Report category boundaries when continuous variables were categorized Pg. 6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses N/A
Discussion		
Key results	18	Summarise key results with reference to study objectives Pgs. 7-8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Pgs. 8-9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Pgs. 7-9
Generalisability	21	Discuss the generalisability (external validity) of the study results Pg. 9
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.