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Breastfeeding and offspring IQ at age 5. Findings from the lifestyle during pregnancy study

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Breastfeeding and offspring IQ at age 5. Findings from the lifestyle during pregnancy study.

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ABSTRACT

Objectives

Breastfeeding is associated with health benefits for both mother and child, but many studies focusing on neurodevelopment have lacked information on important confounders and few randomized trials exist. Our objective was to examine the influence of breastfeeding on child IQ at 5 years of age while taking maternal IQ and home environment into account.

Design, setting, participants and outcome measures

We used data from The Lifestyle During Pregnancy Study, which consists of 1782 mother-child pairs sampled from the prospective observational Danish National Birth Cohort (n=101,042) based on maternal alcohol intake, diet and breastfeeding. Child IQ was assessed at age 5 years by the Wechsler Primary and Preschool Scales of Intelligence-Revised as part of a comprehensive assessment of neurobehavioural development. On the same occasion maternal intelligence was assessed by subtests from the Wechsler Adult Intelligence Scale and Raven's Standard Progressive Matrices. Exposure data on duration of breastfeeding (n=1385) were extracted from telephone interviews conducted when the child was 6 and 18 months. In multivariable linear regression analyses several potential confounders were included, and analyses were weighted by relevant sampling fractions.

Results

Breastfeeding was associated with child IQ at 5 years (p=0.04). Compared to children who were breastfed for one month or less, children who were breastfed for 2-3, 4-6, 7-9 and 10 or more months had 3.06 (95% CI 0.39;5.72), 1.90 (95% CI -0.59;4.38), 3.53 (95% CI 1.18;5.87) and 3.28 (95% CI 0.88;5.67) points higher IQ, respectively. There was no dose-response relation and further analyses indicated that the main difference in IQ was between breastfeeding ≤1 month vs >1 month.

Conclusions

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Breastfeeding was associated with approximately 3 points higher IQ, but there was no evidence of a dose-response relation in this prospective birth cohort, where we were able to adjust for some of the most critical confounders, including maternal intelligence.

Funding

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The funding sources had no role in the study design, interpretation of the data, or publication of the results.

Conflicts of interests statement

All authors declare no competing interests

Data sharing statement

Data are not available for this study due to the data regulations from the relevant cohort responsibles: the Lifestyle During Pregnancy Study and the Danish National Birth Cohort.

Abbreviations

BMI: body mass index

CI: confidence interval

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DNBC: Danish National Birth Cohort

FSIQ: Full Scale IQ

IQ: intelligence quotient

IQR: Interquartile range

LDPS: Lifestyle During Pregnancy Study

SD: standard deviation

WPPSI-R: Wechsler Primary and Preschool Scales of Intelligence-Revised

Article Summary

Strengths and limitations of this study

- Study based on a large population based pregnancy cohort with prospective data assessments
- This study of the association between breastfeeding and child IQ took into account maternal intelligence and home environment, two critical confounders
- Very few women did not breastfeed, so it was not possible to investigate this group separately
- Postnatal factors may have influenced child IQ that we have not been able to take into consideration

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INTRODUCTION

There is firm evidence for the beneficial effects of breastfeeding on a wide range of maternal and child outcomes (1;2) and to ensure healthy growth and development the World Health Organization recommends that all children are exclusively breastfed for the first six months of life, followed by partial breastfeeding (3).

Since the very early observations by Hoefer and Hardy in 1929 of a positive association for breastfeeding with cognitive performance at age 7-13 years (4), a large body of evidence has provided results in this field. Due to difficulties and ethical concerns intrinsic in designing an intervention targeting breastfeeding duration, only few randomized trials exist (5-7). Several observational studies have shown positive associations for duration of breastfeeding with cognitive developmental benefits in childhood (8-14) and in adult life (15-18). Differences in nutrient compositions between human milk and formula have been suggested as one of several potential mechanisms underlying a beneficial effect of breastfeeding on cognitive development (16). In some studies, crude positive associations between breastfeeding and cognitive endpoints were attenuated upon adjustment for potential confounders (19), which has made authors conclude that breastfeeding may be a proxy for home environment or parental practices rather than a causal factor in itself (19-25). However, most of these studies have been relatively small, have had limited details on breastfeeding or have not been able to take into account some of the critical confounders including maternal intelligence (26). A systematic review and meta-analysis in 2013 concluded that breastfeeding is associated with increased performance in intelligence tests in childhood and adolescence of 3.5 points on average, and that maternal IQ, despite being an important confounder, can account only for part of this association (3). While the practical implications of such moderate differences in intelligence tests can be questioned, a recent study from Brazil (18) indicated that a cognitive advantage by breastfeeding was maintained until adulthood mediating effects on life

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outcomes including educational attainment and income even in a setting without strong social patterning of breastfeeding (18;27).

The aim of the present study was to assess, in an observational setting, the influence of breastfeeding on child IQ at 5 years of age while taking maternal IQ and home environment into account in the Lifestyle During Pregnancy Study.

METHODS

The aim of the Lifestyle During Pregnancy Study (LDPS) is to examine the relation between maternal lifestyle during pregnancy and offspring neurodevelopment by the age of 5 years (28). The study is a subsample of the large prospective Danish National Birth Cohort (DNBC) (29). The DNBC recruited >100,000 pregnancies between 1996 and 2002, and data collection instruments included two pregnancy telephone interviews (in gestational weeks 12 and 30 approximately), two telephone interviews postpartum (at 6 and 18 months postpartum), as well as a detailed food frequency questionnaire (FFQ) which was mailed to the women in gestational week 25 and covered the dietary intake in the previous month (30). Information on birth outcome was obtained from the Medical Birth Registry.

The LPDS consists of 3478 mother-child dyads selected from the DNBC with oversampling of pregnant women with moderate weekly alcohol intake, alcohol binge drinkers and women with high vs. low fish intake, iron intake and duration of breastfeeding, respectively (28). A detailed description of the LDPS, including the sampling frame, has been published elsewhere (31;32). In brief, 3478 mother-child pairs from the DNBC were invited to participate in a follow up around the time when the children turned 5 years. Of the selected dyads 1782 mothers and children (51%) took part in a comprehensive three-hour assessment of neurodevelopment focusing on global cognition, specific cognitive functions, and behaviour of the child, and additionally mothers completed tests of adult intelligence (IQ) (31).

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Exclusion criteria for the follow-up in LDPS were multiple pregnancies, inability to speak Danish, impaired hearing or vision of the child likely to compromise the ability to perform the cognitive tests, and congenital disorders likely to cause mental retardation, including trisomy 21 (31). All test procedures were standardised in detail and carried out by 10 trained psychologists. The examinations took place in the four largest cities of Denmark: Copenhagen, Aarhus, Odense and Ålborg (31).

Exposure variables

At both telephone interviews postpartum, when the child was 6 and 18 months, respectively, mothers answered questions about breastfeeding. At the 6 months' interview they reported whether they had never breastfed, were currently breastfeeding or had stopped breastfeeding. Those who were no longer breastfeeding reported the child's age when daily breastfeeding stopped. At 18 months postpartum mothers were asked whether they had breastfed their child beyond 6 months, and if so, whether they were still breastfeeding; if not, they reported the child's age at discontinuation of breastfeeding. Combining the answers from these two data collection instruments we constructed a measure of duration of any breastfeeding, not distinguishing between exclusive and supplemented breastfeeding.

Outcome variables

We used child IQ assessed at the follow up examination at age 5 with the Wechsler Primary and Preschool Scales of Intelligence-Revised (WPPSI-R) (33). The WPPSI-R is one of the most widely used standardized tests of intelligence for children of 2-7 years of age. In its full form the test battery for WPPSI-R comprises 5 verbal subtests and 5 performance (non-verbal) subtests from which verbal IQ, performance IQ, and full scale (FS) IQs are derived. The length of the examination was accommodated to match the age of the study participants (5 years), and therefore a

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short form of the WPPSI-R was used, which included three verbal (Arithmetic, Information and Vocabulary) and three performance subtests (Block Design, Geometric Design and Object Assembly). Standard procedures were used to prorate IQs from the shortened forms of the tests (34), and since no Danish WPPSI-R norms were available at the time of the study Swedish norms were used to derive scaled scores and IQs (35). The use of Swedish norms might cause a slight shift in the IQ distribution, and the theoretical mean of 100 (SD 15) cannot necessarily be expected in this sample. However, as the analyses conducted in this study are internal comparisons across exposure, a systematic shift in the IQ distribution should not affect our results.

Mother-child pairs available for analysis

Out of the total number of participants in the LDPS (n=1782), the study sample for these analyses consisted of those 1772 (99.4%) mother-child dyads in the LDPS with full outcome data on the WPPSI-R. Full information on breastfeeding was available for 1385 (78.2%) of these as lined out in **figure 1**.

Covariates

We used information from the prenatal telephone interviews, a questionnaire at the 5-year follow-up examination, and from the Medical Birth Registry and Central Person Registry to define potential confounders that we included in our analyses as covariates. Maternal IQ was assessed at the follow-up examination by two verbal subtests (information and vocabulary) from the Wechsler Adult Intelligence Scale (36) and non-verbal IQ by the Raven's Standard Progressive Matrices (37). Raw scores of each test were standardised based on the results from the full sample, and were weighted equally into a combined score which was subsequently restandardised to a scale with a mean of 100 and SD of 15.

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We defined the covariates as follows: parity (0, 1, ≥2); prenatal maternal smoking (yes, no); maternal pre-pregnancy body mass index (BMI) (weight(kg)/height(m)^2); parental education (average educational length in years for the parents or of mother if that of the father was unavailable); marital status (single during pregnancy or at 5 year follow up, married/cohabitating at both); postnatal parental smoking (yes if mother or father smoked in the home, no if otherwise); maternal average alcohol intake in pregnancy (0, 1-4, 5-8, ≥9 drinks/week); child health status (any medical conditions or regular medications present that might influence test performance such as epilepsy, syndromes including Morpheus syndrome, Neurofibromatosis Recklinghausen, congenital toxoplasmosis and myxedema; and/or usage of medicine for asthma and allergy, ADHD (methylphenidate), epilepsy and respiratory conditions, none such conditions present); dichotomized home environment index (presence of two or more of the following seven conditions: not living with a biological parent, changes in caregiver, day care before age 3, 14+ days of separation from the parents, breakfast irregularity, maternal depression and parental alcohol use above the maximum recommended level by the Danish Health Authority at the time of follow-up of 14 (women) or 21 (men) drinks/week); maternal age (in years); maternal IQ (score), sex of child (girl, boy) and age of child at the follow-up examination.

The 10 trained psychologists who carried out the testing of the study participants were blinded to the child's exposure status, and we took tester differences into account by the inclusion of a categorical variable with 10 levels in the statistical analyses.

Statistical analyses

We used the median (interquartile range (IQR)) to describe skewed variables; mean (SD) for normally distributed variables; and n/percentage for categorical variables.

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The exposure variable, breastfeeding duration, was categorized as any breastfeeding ≤1 month, 2-3 months, 4-6 months, 7-9 months or ≥10 months. A linear association was tested by entering months of breastfeeding duration into the models as a continuous variable

Furthermore, for supplementary analysis we dichotomized the exposure variable in order to examine the difference in FSIQ according to duration of any breastfeeding ≤1 month vs. >1 month.

We compared WPPSI-R FSIQ scores across exposure levels by using multivariable linear regression models to obtain β estimates and corresponding 95% confidence intervals (CIs). In our multivariable analyses we adjusted for potential confounders in two steps: maternal IQ, parental education, maternal smoking during pregnancy, age of child at the follow-up examination and tester were considered core confounders, and we considered this our main analysis.; in a more extensively adjusted model we furthermore included the following potential confounders: maternal age, maternal marital status, parity, sex of child, maternal prepregnancy BMI, postnatal parental smoking, health index, home environment and maternal average alcohol intake during pregnancy.

Supplementary to this, a non-linear relation between duration of breastfeeding and child FSIQ was tested comparing a linear model to a model using restricted cubic splines with 3 knots (38).

Due to the oversampling of study participants according to certain behavioural exposures, as described above (moderate alcohol use, binge drinking, fish, iron intake and duration of breastfeeding) all analyses were weighted by sampling probabilities.

For the 1385 mother-child pairs with data on exposures and outcome, data on child's age at testing and testing psychologist was complete, whereas information on other covariates was missing for approximately 8% of participants (ranging from 7.9% for maternal smoking during pregnancy to 9.5% for maternal prepregnancy BMI). We substituted missing covariate values by multiple imputation using the standard procedure PROC MI in SAS, which utilizes all the information available and imputes 5 different datasets based on which we calculated 5 different sets of effect and variance estimates, and subsequently used the procedure PROC MIANALYZE to compute a

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combined estimate and standard error for each regression coefficient in our analyses. Results from alternative analytic strategies using complete case and substitution of missing values with mean/mode imputation did not alter our conclusions.

All analyses were carried out using SAS statistical software (version 9.4, SAS Institute, Cary, North Carolina, USA), and all tests were two-sided with statistical significance set to $p<0.05$.

Supplementary analyses

We suspected gestational age and birth weight to be intermediate variables, and they were therefore not included in our main models to avoid overadjustment. We stratified by child sex, in order to examine potential sex specific effects. We also conducted separate analyses for performance IQ and verbal IQ, respectively. Finally, we analysed the three IQ outcomes dichotomised, using the sample mean minus one SD for the relevant IQ score (full scale IQ, performance IQ, or verbal IQ) as a cut-off score for subnormal test performance, by logistic regression models.

Participants gave informed consent on behalf of themselves and their children. The study was approved by the DNBC Board of Directors, the DNBC Steering committee, the regional Ethics Committee, the Danish Data Protection Agency, and the CDC Institutional Review Board.

RESULTS

The children in the LDPS were 60-64 months of age at follow up (mean 5.2, range 5.0-5.3 years), and 52% of the children were boys (32).

Women who breastfed for less than one month compared to longer periods were generally younger, they were more likely to be nulliparous, have higher BMI, to have been smokers during pregnancy or to have their children be exposed to tobacco smoke postnatally, have lower IQ, shorter education and lower birthweight of the index child (**table 1**).

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Child IQ at the 5-year examination was directly associated with maternal IQ (Spearman r=0.29, p<0.0001), parental education, birthweight and gestational age, whereas associations were inverse for parity, prepregnancy BMI, smoking during pregnancy and postnatal smoke exposure. Mean IQ was slightly higher for girls compared with boys (data not shown).

Duration of breastfeeding was associated with child IQ at 5 years, even after adjustment for core confounders which included maternal IQ (p from categorical test 0.03) (**table 2**). Compared to children who were breastfed one month or less, children who were breastfed for 2-3, 4-6, 7-9 and 10 or more months had 3.06 (95% CI 0.39;5.72), 1.90 (95% CI -0.59;4.38), 3.53 (95% CI 1.18;5.87) and 3.28 (95% CI 0.88;5.67) points higher IQ, respectively. These results remained very similar after more extensive adjustment of potential confounders. Furthermore, the estimates for 2-3, 4-6, 7-9, ≥10 months breastfeeding groups were not statistically different from each other (p=0.21), indicating that there was no dose-response, but that the difference in FSIQ lies between those breastfed ≤1 month vs >1 month. Dichotomizing the breastfeeding variable by duration ≤1 month vs >1 month resulted in a 2.98 (95 CI 0.86;5.11) point lower FSIQ for children who were breastfed ≤1 month vs >1 month. When analysing duration of breastfeeding as a continuous variable, the association was statistically significant only in the unadjusted analyses (p value for linear trend unadjusted <0.0001, adjusted = 0.68) We subsequently tested for a non-linear association using restricted cubic splines, which provided significantly better fit compared to the linear model (p=0.02), and the model with splines was significantly different from the null (p for non-linearity =0.03).

Adjustment for gestational age and birthweight in supplementary analyses did not alter our study results. When we analysed subscales of IQ, associations were strongest for performance IQ; for verbal IQ effect estimates went in the same direction as for full scale IQ, but were attenuated and not consistently statistically significant across the different adjustment models (data not shown).

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When we stratified for child sex, overall associations were the same and remained statistically significant for both boys and girls, even if confidence intervals widened considerably and effect estimates for the specific breastfeeding groups fluctuated between boys and girls.

Finally, analyses using dichotomized IQ measures indicated associations in the same direction as the main analyses, but confidence intervals were wide, and associations were generally not statistically significant.

DISCUSSION

In this relatively large study population which was sampled from the Danish National Birth Cohort we investigated associations for duration of breastfeeding with child IQ at age 5 years assessed with the WPPSI-R by a team of trained psychologists. We saw an approximate 3 point difference between those who were breastfed for one month or less compared to those who were breastfed longer. The LDPS oversampled mothers with moderate alcohol intake in pregnancy, which we adjusted for by weighing all analyses by the relevant sampling fractions. Furthermore, maternal alcohol use was not strongly associated with poorer performance at 5 years (32;39;40). We therefore believe it to be unlikely that any of the associations we observe are due to selection bias caused by the sampling in the LDPS.

Our results are in support of the previous studies that have shown beneficial associations for breastfeeding with cognitive measures in children (9-12;41;42). It furthermore adds to the current body of literature, since we were able to take maternal IQ into account, had detailed information on breastfeeding, clinical assessment of child IQ by a validated tool, as well as a relatively large sample size. The observed difference in IQ score in our analyses corresponds well with the results of a recent meta-analysis which reported a pooled effect of 3.5 IQ points associated with breastfeeding, and a slightly smaller difference of 2.2 IQ points in studies that were able to adjust

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for maternal intelligence (3). According to Jacobson et al the contribution of maternal intelligence to the child's cognitive ability is both genetic and through a more stimulating rearing environment (43). Furthermore, studies that fail to include maternal intelligence when attempting to show that the link between breastfeeding and cognitive outcome is not attributable to social factors underestimate a critically important factor (24;43). Our results thus point to some effect of breastfeeding on child IQ over and above that of parent practices and heredity.

We found no clear dose response relation of breastfeeding duration with child cognitive development in our data; rather, our results point to a marked difference in IQ of more than 3 points between children who are breastfeed for a short period of one month or less compared to those who are breastfed longer. This stands somewhat in contrast with a meta-analysis from 1999 (8) and a more recent systematic literature review (1) which concluded that cognitive developmental benefits increased with duration of breastfeeding. In our data such a relation was present in the unadjusted analysis only, and we can therefore speculate that effect patterns and sizes may differ between studies depending on other variables that influence IQ, or that previous studies have not investigated such threshold effects of breastfeeding.

In our study sample we categorized the shortest duration as ≤1 month, since very few women reported breastfeeding duration shorter than this, reflecting that by far the majority of mothers in Denmark choose to breastfeed their children. Adding to the difficulty of obtaining an exposure group with shorter duration of breastfeeding is the fact, that women who from the beginning choose not to breastfeed may be different from those who do breastfeed; for example, women who rely on medication for various reasons may choose not to breastfeed because of concerns that medication in the breastmilk may harm the infant. This may explain the recent results from the Dutch Generation R Study (n=3761), which compared non-verbal IQ scores among those 'never breastfed' with different durations of breastfeeding, and found a positive association that was, however, attenuated

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and no longer statistically significant after adjustment for maternal IQ and several potential confounders (20). Another explanation may be that they assessed non-verbal intelligence by two sub-tests of a Dutch non-verbal IQ test. We used the WPPSI-R, for which reliability coefficients for the IQs for the present age group are very high (0.90-0.96), although they are likely to be somewhat lower when IQs are based on only three verbal and three performance subtests (35).

Our study sample was originally selected to investigate effects of low to moderate alcohol intake on child development, however, this did not seem to inflict on breastfeeding behaviour, since rates of breastfeeding in our sample were similar to rates in the DNBC overall. The differences between women who breastfeed or not can only be addressed entirely in a randomized controlled trial, such as the large PROBIT-study in Belarus, where randomization of clinics and hospitals to breastfeeding promotion resulted in a substantial increase both in breastfeeding exclusivity and duration of any breastfeeding. At age 6.5 years children from the PROBIT-study were followed up (n=13889), and children from the intervention arm scored 7.5 points higher on verbal IQ from the Wechsler Abbreviated Scales of Intelligence, providing strong evidence for a beneficial effect of prolonged breastfeeding on children's cognitive development (5). Such effects may have a long term impact, as suggested by a Danish observational study, which showed a beneficial association between duration of breastfeeding and adult intelligence in two non-overlapping samples (n=973 men and women, n=2280 men, respectively) (16). According to Huang et al subsequent schooling and other socialization experiences during adolescence do not eliminate the breastfeeding gap that appears in very early childhood (44). Furthermore, recent studies have found that the effects of breastfeeding may translate into substantial educational differences in adult life (18) and economic gains to society (45).

Previous studies have suggested three different mechanisms underlying such associations: differences in nutrient compositions between human milk and formula, interplay around the feeding situation, and unidentified factors that correlate with both breastfeeding and neurodevelopment,

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including residual confounding (16). With respect to differences in nutrient content, long chained polyunsaturated n-3 fatty acids, and docosahexaenoic acid (DHA) in particular, may be involved. DHA is deemed an essential fatty acid, because it cannot be intrinsically produced and must hence be provided by the diet, with fatty fish as the main source. Results from the Danish National Birth Cohort showed both breastfeeding and maternal fish intake to be independently associated with developmental milestones in the young offspring (46).

It is a main strength of our study that we were able to include the most important confounders: maternal intelligence, parental education and home environment, which in separate analyses have been shown to account for 19-29% of the variance in IQ (47;48), also in analyses conducted within the LDPS (49). Additional strengths to our study include the relatively large number of mother-children pairs in our sample, and the fact that child IQ was assessed by a state of the art clinical intelligence test with high reliability coefficients for IQ for the present age group.

The main limitations of our study are the different possible sources of bias inherent in an observational study design. As is the case in most large cohort studies, participants in the DNBC were generally better off and more health conscious compared to the overall Danish population of pregnant women. However, according to a study by Nøhr et al this selection did not result in biased estimates for selected aetiological associations (50). Furthermore, at the time the women in the DNBC reported on breastfeeding duration they were not aware of the outcome under study, making it less likely that their reporting was affected by this.

Associations tended to be attenuated with increasing number of covariates in our analyses, suggesting unadjusted confounding or sparse data bias (51). As is always the case in observational studies, we cannot exclude that our results are flawed by residual confounding or confounding by factors not accounted for in our analyses. Furthermore, there may be postnatal factors influencing

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child IQ that we have not been able to take into consideration; however, these may not act as true confounders but rather as intermediates, in which case they should not be included in the analyses. Current recommendations from the World Health Organization are that women should breastfeed exclusively for the first six months. Our finding of a 3 point difference in IQ associated with any duration of breastfeeding longer than one month is in support of current recommendations, and is even a relaxed message to mothers who struggle with exclusive breastfeeding. Seen from a public health perspective a difference of 3 IQ points must be considered substantial, and smaller effects have previously led to quite conservative precautionary recommendations, for example with respect to adverse effects of maternal exposure to environmental toxicants (52).

In conclusion, in this large sample with high quality assessment of child IQ, we found support of a beneficial association for breastfeeding with child IQ at 5 years of age, while adjusting for maternal IQ and parental education, which only few previous studies have been able to do. Taking the necessary precautions that our results may reflect residual confounding, our findings support current recommendations with respect to breastfeeding in relation to cognitive development of the child.

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Author Contributions

The authors' responsibilities were as follows:

SFO and MS designed research; ELM, UK, SFO, TIH and MS provided essential materials and conducted research; MS and TIH performed statistical analysis; MS wrote paper; MS had primary responsibility for final content. All authors read and approved the final manuscript. All authors had full access to study data. None of the authors had a conflict of interest.

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Table 1: Study participants in the Lifestyle during pregnancy study distributed by maternal and child characteristics and by breastfeeding duration (n=1385)

			Breastfeeding duration					
	Overall	**p[1]**	**≤1 month**	**2-3 months**	**4-6 months**	**7-9 months**	**≥10 months**	**p[1]**
Maternal age (years)[2]	30.9 (4.4)	0.06	30.9 (4.5)	29.9 (4.4)	30.1 (4.2)	30.9 (4.1)	32.0 (4.3)	<0.0001
Parity[2]		<0.0001						0.0003
Nulliparous	863 (48.7)		80 (57.6)	82 (56.2)	122 (49.0)	194 (49.0)	198 (43.5)	
1 child	604 (31.1)		41 (29.5)	50 (34.3)	88 (35.3)	142 (35.9)	146 (32.1)	
≥2 children	304 (17.2)		18 (13.0)	14 (9.6)	39 (15.7)	60 (15.2)	111 (24.4)	
Prepregnancy BMI (kg/m^2)[2]	22.7 (4.4)	0.99	22.8 (4.1)	23.3 (4.7)	22.7 (4.7)	22.6 (4.0)	22.2 (4.3)	<0.0001
Marital status[2]		0.8						0.3
Single	49 (2.8)		4 (2.9)	2 (1.4)	11 (4.4)	7 (1.8)	13 (2.9)	
Cohabiting with partner	1722 (97.2)		135 (97.1)	144 (98.6)	238 (95.6)	389 (98.2)	442 (97.1)	
Alcohol, drinks/wk in pregnancy[2]	0.5 (2.0)	0.4	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.3
Smoking in pregnancy[2]		0.5						<0.0001
Yes	568 (32.1)		54 (38.9)	66 (45.2)	91 (36.6)	102 (25.8)	131 (28.8)	
No	1203 (67.9)		85 (61.2)	80 (54.8)	158 (63.5)	294 (74.2)	324 (71.2)	
Postnatal smoking[2]		0.03						<0.0001
Yes	583 (32.9)		51 (36.7)	74 (50.7)	97 (39.0)	98 (24.8)	129 (28.4)	
No	1188 (67.1)		88 (63.3)	72 (49.3)	152 (61.0)	298 (75.3)	326 (71.7)	
Maternal IQ [2]	100.0 (14.9)	0.1	100.4 (14.9)	94.3 (14.8)	97.1 (13.8)	102.2 (14.0)	103.3 (14.9)	<0.0001
Parental education (years)[2]	13.0 (3.0)	0.03	13.0 (3.5)	12.3 (3.0)	12.5 (2.5)	13.0 (2.5)	13.5 (2.5)	<0.0001
Child sex[2]								0.5
Girls	849 (47.9)	0.9	74 (53.2)	66 (45.2)	115 (46.2)	178 (45.0)	221 (48.6)	
Boys	922 (52.1)		65 (46.8)	80 (54.8)	134 (53.8)	218 (55.1)	234 (51.4)	
Birthweight (g)[2]	3602 (516)	0.2	3615 (529)	3456 (524)	3589 (487)	3601 (490)	3649 (513)	0.007
Gestational age (days)[2]	280.5 (10.5)	0.9	280.8 (10.1)	278.7 (12.1)	280.0 (10.6)	281.1 (10.3)	280.5 (9.8)	0.3

[1] p-value from chi^2 test (categorical variables: parity, marital status, smoking in pregnancy, postnatal smoking, child sex), F-test one way anova (normally distributed variables: maternal age, maternal IQ, birthweight, gestational age), or Kruskal-Wallis test (non-normally distributed variables: prepregnancy BMI, alcoholic drinks/week in pregnancy, parental education)

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[2] Values are mean (SD) (for normally distributed variables: maternal age, maternal IQ, birthweight, gestational age), median (IQR) (for non-normally distributed variables: prepregnancy BMI, alcoholic drinks/week in pregnancy, parental education) or n (%) (for categorical variables: parity, marital status, smoking in pregnancy, postnatal smoking, child sex)

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Table 2: Full scale IQ among 1385 children from the Lifestyle During Pregnancy Study in association with breastfeeding duration

		Crude		Adjusted for core confounders[2]		Adjusted for all potential confounders[2]	
Breastfeeding	Mean score Full scale IQ[1]	Mean difference	95% CI	Mean difference	95% CI	Mean difference	95% CI
	101.93						
Pr. month breastfeeding		0.33	(0.19; 0.47)	0.04	(-0.10; 0.18)	0.07	(-0.08;0.21)
		<0.0001[3]		*0.58*[3]		*0.37*[3]	
<=1 month	98.84						
2-3 months		3.92	(1.13;6.71)	3.06	(0.39;5.72)	3.24	(0.61;5.86)
4-6 months		4.37	(1.87;6.87)	2.03	(-0.38;4.44)	1.70	(-0.69;4.08)
7-9 months		6.61	(4.21;9.01)	3.53	(1.18;5.87)	3.36	(1.06;5.66)
≥10 months		7.85	(5.46;10.25)	3.28	(0.88;5.67)	3.41	(1.02;5.80)
		<0.0001[4]		*0.03*[4]		*0.02*[4]	

1. Wechsler Preschool and Primary Scale of Intelligence-Revised
2. Covariate models; core confounders: Maternal IQ, parental education, smoking during pregnancy, child's age at testing, testing psychologist. All potential confounders: core + maternal age, maternal marital status, maternal parity, sex of child, maternal prepregnant BMI, postnatal parental smoking, health index, home environment, maternal average alcohol intake during pregnancy.
3. *P*-value for the hypothesis of no trend in IQ-scores across levels of exposure
4. *P*-value for the hypothesis of no difference in IQ-scores across levels of exposure, categorical test

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Figure legend

Figure 1
Overview of the study sample from the Lifestyle During Pregnancy Study

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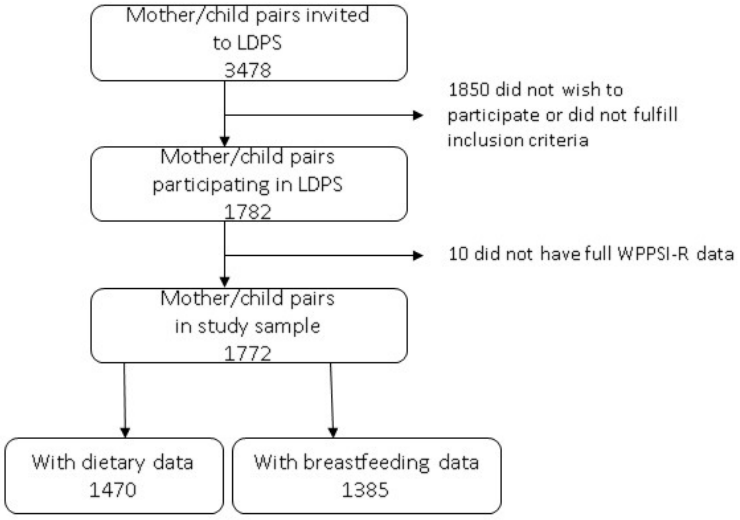

FIgure 1

200x120mm (96 x 96 DPI)

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STROBE Statement—Checklist of items that should be included in reports of ***cohort 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 X – p. 4
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found X – p. 4-5
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported X – p. 6-7
Objectives	3	State specific objectives, including any prespecified hypotheses X – p. 7
Methods		
Study design	4	Present key elements of study design early in the paper X – p. 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection X – p. 7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up X – p. 7-8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable X – p. 8-10
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 X – p. 7-10
Bias	9	Describe any efforts to address potential sources of bias X – p. 9-12
Study size	10	Explain how the study size was arrived at X – p. 9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why X – p. 8-10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding X – p. 10-12
		(*b*) Describe any methods used to examine subgroups and interactions X – p. 12
		(*c*) Explain how missing data were addressed X – p. 12
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses X – p. 12
Results		

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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 X – p. 9
		(b) Give reasons for non-participation at each stage X – p. 9
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders X – p. 12
		(b) Indicate number of participants with missing data for each variable of interest X – p. 11
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time X – p. 12-13
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 X – p. 13-14, Table 2
		(*b*) Report category boundaries when continuous variables were categorized X – Table 1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses X – p. 13-14
Discussion		
Key results	18	Summarise key results with reference to study objectives X – p. 14
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 X – p. 15,18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence X – p. 15-18
Generalisability	21	Discuss the generalisability (external validity) of the study results X – p. 15-16
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 X – p. 1-2

*Give information separately for exposed and unexposed groups.

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 http://www.strobe-statement.org.

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Is breastfeeding associated with offspring IQ at age 5? Findings from prospective cohort: lifestyle during pregnancy study

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Is breastfeeding associated with offspring IQ at age 5? Findings from prospective cohort: lifestyle during pregnancy study.

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ABSTRACT

Objectives

Breastfeeding is associated with health benefits for both mother and child, but many studies focusing on neurodevelopment have lacked information on important confounders and few randomized trials exist. Our objective was to examine the influence of breastfeeding on child IQ at 5 years of age while taking maternal IQ and other relevant factors into account.

Design, setting, participants and outcome measures

We used data from The Lifestyle During Pregnancy Study, consisting of 1782 mother-child pairs sampled from the Danish National Birth Cohort (n=101,042). Child IQ was assessed at age 5 years by the Wechsler Primary and Preschool Scales of Intelligence-Revised. On the same occasion maternal intelligence was assessed by Wechsler Adult Intelligence Scale and Raven's Standard Progressive Matrices. Exposure data on duration of breastfeeding (n=1385) were extracted from telephone interviews conducted when the child was 6 and 18 months, and analyses were weighted by relevant sampling fractions.

Results

In multivariable linear regression analyses adjusted for potential confounders breastfeeding was associated with child IQ at 5 years (categorical chi^2 test for overall association p=0.03). Compared to children who were breastfed ≤1 month, children breastfed for 2-3, 4-6, 7-9 and 10 or more months had 3.06 (95% CI 0.39;5.72), 1.90 (95% CI -0.59;4.38), 3.53 (95% CI 1.18;5.87) and 3.28 (95% CI 0.88;5.67) points higher IQ after adjustment for core confounders, respectively. There was no dose-response relation and further analyses indicated that the main difference in IQ was between breastfeeding ≤1 month vs >1 month.

Conclusions

Breastfeeding duration of 1 month or shorter compared to longer periods was associated with approximately 3 points higher IQ, but there was no evidence of a dose-response relation in this

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prospective birth cohort, where we were able to adjust for some of the most critical confounders, including maternal intelligence.

Article Summary

- Study based on a large population based pregnancy cohort with prospective data assessments
- This study of the association between breastfeeding and child IQ took into account maternal intelligence and home environment, two critical confounders
- Very few women did not breastfeed (n=6 / 0.4%), so it was not possible to investigate this group separately
- Postnatal factors may have influenced child IQ that we have not been able to take into consideration

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Competing Interests

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All authors declare no competing interests

Data sharing statement

Data are not available for this study due to the data regulations from the relevant cohort responsibles: the Lifestyle During Pregnancy Study and the Danish National Birth Cohort.

Abbreviations

BMI: body mass index

CI: confidence interval

DNBC: Danish National Birth Cohort

FSIQ: Full Scale IQ

IQ: intelligence quotient

IQR: Interquartile range

LDPS: Lifestyle During Pregnancy Study

SD: standard deviation

WPPSI-R: Wechsler Primary and Preschool Scales of Intelligence-Revised

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INTRODUCTION

There is firm evidence for the beneficial effects of breastfeeding on a wide range of maternal and child outcomes [1 2] and to ensure healthy growth and development the World Health Organization recommends that all children are exclusively breastfed for the first six months of life, followed by partial breastfeeding [3].

Since the very early observations by Hoefer and Hardy in 1929 of a positive association for breastfeeding with cognitive performance at age 7-13 years [4], a large body of evidence has provided results in this field. Due to difficulties and ethical concerns intrinsic in designing an intervention targeting breastfeeding duration, few randomized trials exist [5-7]. These have shown beneficial effects of breastfeeding in relation with child neurodevelopmental outcomes, and one of these indicated that exclusive breastfeeding may not confer superior effects compared to breastfeeding and complementary foods introduced at 4 months of age [7]. Several observational studies have shown positive associations for duration of breastfeeding with cognitive developmental benefits in childhood [8-16] and in adult life [17-20]. Differences in nutrient compositions between human milk and formula have been suggested as one of several potential mechanisms underlying a beneficial effect of breastfeeding on cognitive development [18]. In some studies, crude positive associations between breastfeeding and cognitive endpoints were attenuated upon adjustment for potential confounders, including socioeconomic background [21-25], which has made authors conclude that breastfeeding may be a proxy for home environment or parental practices rather than a causal factor in itself [21-27]. However, many studies have been relatively small (n<500), have had limited details on breastfeeding or have not been able to take into account some of the critical confounders including maternal intelligence [9 12 26]. A systematic review and meta-analysis in 2013 concluded that breastfeeding is associated with increased performance in intelligence tests in childhood and adolescence of 3.5 points on average, and that maternal IQ, despite being an important confounder, can account only for part of this association [3]. While the practical implications of such moderate

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differences in intelligence tests can be questioned, a recent study from Brazil [20] indicated that a cognitive advantage by breastfeeding was maintained until adulthood mediating effects on life outcomes including educational attainment and income even in a setting without strong social patterning of breastfeeding [20 28].

The aim of the present study was to assess, in an observational setting, the influence of breastfeeding on child IQ at 5 years of age while taking maternal IQ and home environment into account in the Lifestyle During Pregnancy Study.

SUBJECTS AND METHODS

The aim of the Lifestyle During Pregnancy Study (LDPS) is to examine the relation between maternal lifestyle during pregnancy and offspring neurodevelopment by the age of 5 years [29]. The study is a subsample of the large prospective Danish National Birth Cohort (DNBC) [30]. The DNBC recruited >100,000 pregnant women between 1996 and 2002, and data collection instruments included two pregnancy telephone interviews (in gestational weeks 12 and 30 approximately), two telephone interviews postpartum (at 6 and 18 months postpartum), as well as a detailed food frequency questionnaire (FFQ) which was mailed to the women in gestational week 25 and covered the dietary intake in the previous month [31]. Information on birth outcome was obtained from the Medical Birth Registry.

The LPDS consists of 3478 mother-child dyads selected from the DNBC with oversampling of pregnant women with moderate weekly alcohol intake, alcohol binge drinkers and women with high vs. low fish intake, iron intake and duration of breastfeeding, respectively [29]. A detailed description of the LDPS, including the sampling frame, has been published elsewhere [29 32]. In brief, 3478 mother-child pairs from the DNBC were invited to participate in a follow up around the time when the children turned 5 years. Of the selected dyads 1782 mothers and children (51%) took part in a comprehensive three-hour assessment of neurodevelopment focusing on global cognition, specific

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cognitive functions, and behaviour of the child, and additionally mothers completed tests of adult intelligence (IQ) [29].

Exclusion criteria for the follow-up in LDPS were multiple pregnancies, inability to speak Danish, impaired hearing or vision of the child likely to compromise the ability to perform the cognitive tests, and congenital disorders likely to cause mental retardation, including trisomy 21 [29]. All test procedures were standardised in detail and carried out by 10 trained psychologists. The examinations took place in the four largest cities of Denmark: Copenhagen, Aarhus, Odense and Ålborg [29].

Exposure variables

At both telephone interviews postpartum, when the child was 6 and 18 months, respectively, mothers answered questions about breastfeeding. At the 6 months' interview they reported whether they had never breastfed, were currently breastfeeding or had stopped breastfeeding. Those who were no longer breastfeeding reported the child's age when daily breastfeeding stopped. At 18 months postpartum mothers were asked whether they had breastfed their child beyond 6 months, and if so, whether they were still breastfeeding; if not, they reported the child's age at discontinuation of breastfeeding. Combining the answers from these two data collection instruments we constructed a measure of duration of any breastfeeding, not distinguishing between exclusive and supplemented breastfeeding.

Outcome variables

We used child IQ assessed at the follow up examination at age 5 with the Wechsler Primary and Preschool Scales of Intelligence-Revised (WPPSI-R) [33]. The WPPSI-R is one of the most widely used standardized tests of intelligence for children of 2-7 years of age. In its full form the test battery for WPPSI-R comprises 5 verbal subtests and 5 performance (non-verbal) subtests from

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which verbal IQ, performance IQ, and full scale (FS) IQs are derived. The length of the examination was accommodated to match the age of the study participants (5 years), and therefore a short form of the WPPSI-R was used, which included three verbal (Arithmetic, Information and Vocabulary) and three performance subtests (Block Design, Geometric Design and Object Assembly). Standard procedures were used to prorate IQs from the shortened forms of the tests [34], and since no Danish WPPSI-R norms were available at the time of the study Swedish norms were used to derive scaled scores and IQs [35]. The use of Swedish norms might cause a slight shift in the IQ distribution, and the theoretical mean of 100 (SD 15) cannot necessarily be expected in this sample. However, as the analyses conducted in this study are internal comparisons across exposure, a systematic shift in the IQ distribution should not affect our results.

Mother-child pairs available for analysis

Out of the total number of participants in the LDPS (n=1782), the study sample for these analyses consisted of those 1772 (99.4%) mother-child dyads in the LDPS with full outcome data on the WPPSI-R. Full information on breastfeeding was available for 1385 (78.2%) of these as lined out in **figure 1**.

Covariates

We used information from the prenatal telephone interviews, a questionnaire at the 5-year follow-up examination, and from the Medical Birth Registry and Central Person Registry to define potential confounders that we included in our analyses as covariates. Maternal IQ was assessed at the follow-up examination by two verbal subtests (information and vocabulary) from the Wechsler Adult Intelligence Scale [36] and non-verbal IQ by the Raven's Standard Progressive Matrices [37]. Raw scores of each of the two subtests (information and vocabulary) were standardised based on the results from the full sample. Subsequently scores from each of the two subtests were weighted

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equally into one combined score, and this combined score was then restandardised to a scale with a mean of 100 and SD of 15.

We defined the covariates as follows: parity (0, 1, ≥2 previous births); prenatal maternal smoking (yes, no); maternal pre-pregnancy body mass index (BMI) (weight(kg)/height(m)^2); parental education (average educational length in years for the parents or of mother if that of the father was unavailable); marital status (single during pregnancy or at 5 year follow up, married/cohabitating at both); postnatal parental smoking (yes if mother or father smoked in the home, no if otherwise); maternal average alcohol intake in pregnancy (0, 1-4, 5-8, ≥9 drinks/week); an index of the child's health status (dichotomized as normal or suboptimal in the presence of any illness, diseases, handicaps and/or medication with potential influence on test performance); an index of the quality of postnatal home environment (dichotomized as normal or suboptimal in the presence of two or more of the following seven conditions: not living with a biological parent, changes in caregiver, day care for more than 8 hours/day before age 3, 14+ days of separation from the parents, breakfast irregularity, maternal depression and parental alcohol use above the maximum recommended level by the Danish Health Authority at the time of follow-up of 14 (women) or 21 (men) drinks/week); maternal age at pregnancy (in years); maternal IQ (score), sex of child (girl, boy) and age of child at the follow-up examination. Gestational age was calculated from the last menstrual period provided by the mother at study recruitment (gestation week 6-10) or from the expected date of delivery provided by the woman during the second telephone interview (gestation week 30), which was most often based on ultrasound results [38]. The date of birth was extracted from the Central Person Registry, and the midwife who attended the child's birth recorded birth weight which we extracted from the Medical Birth Registry and calculated sex-specific weight *z*-score at birth on the basis of published reference data [39 40].

The 10 trained psychologists who carried out the testing of the study participants were blinded to the child's exposure status, and we took tester differences into account by the inclusion of a

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categorical variable with 10 levels in the statistical analyses. In a sensitivity analysis tester was taken into consideration in the analysis by a random effect.

Statistical analyses

We used the median (interquartile range (IQR)) to describe skewed variables; mean (SD) for normally distributed variables; and n/percentage for categorical variables.

The exposure variable, breastfeeding duration, was examined in relation to child IQ both as a continuous variable (breastfeeding duration in months), and categorized as any breastfeeding ≤1 month, 2-3 months, 4-6 months, 7-9 months or ≥10 months. When analyzing associations with continuous measures *p*-values from F tests (type II) are presented, for associations with categorical measures *p*-values from chi^2 test (type III) are presented.

Furthermore, for supplementary analysis we dichotomized the exposure variable in order to examine the difference in FSIQ according to duration of any breastfeeding ≤1 month vs. >1 month, and ≤6 months vs. >6 months.

We present mean differences in WPPSI-R FSIQ scores across exposure levels by using multivariable linear regression models to obtain β estimates and corresponding 95% confidence intervals (CIs). In our multivariable analyses we adjusted for potential confounders in two steps: maternal IQ, parental education, maternal smoking during pregnancy, age of child at the follow-up examination and tester were considered core confounders, and we considered this our main analysis.; in a more extensively adjusted model we furthermore included the following potential confounders: maternal age, maternal marital status, parity, sex of child, maternal prepregnancy BMI, postnatal parental smoking, health index, home environment and maternal average alcohol intake during pregnancy. Supplementary to this, a non-linear relation between duration of breastfeeding and child FSIQ was tested comparing a linear model to a model using restricted cubic splines with 3 knots placed at the 25th, 50th and 75th percentiles [41].

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Due to the oversampling of study participants according to certain behavioural exposures, as described above (moderate alcohol use, binge drinking, fish, iron intake and duration of breastfeeding) all analyses were weighted by sampling fractions to account for the complex stratified sampling design [32].

For the 1385 mother-child pairs with data on exposures and outcome, data on child's age at testing and testing psychologist was complete, whereas information on other covariates was missing for approximately 8% of participants (ranging from 7.9% for maternal smoking during pregnancy to 9.5% for maternal prepregnancy BMI). We substituted missing covariate values by multiple imputation using the standard procedure PROC MI in SAS, which utilizes all the information available and imputes 5 different datasets based on which we calculated 5 different sets of effect and variance estimates, and subsequently used the procedure PROC MIANALYZE to compute a combined estimate and standard error for each regression coefficient in our analyses. Results from alternative analytic strategies using complete case and substitution of missing values with mean/mode imputation did not alter our conclusions.

All analyses were carried out using SAS statistical software (version 9.4, SAS Institute, Cary, North Carolina, USA), and all tests were two-sided with statistical significance set to $p<0.05$.

Supplementary analyses

To avoid overadjustment gestational age and birth weight *z*-score were not included in our main models. As supplementary analysis we furthermore excluded those mother-child pairs who never initiated breastfeeding (n=6). We stratified by child sex, in order to examine potential sex specific effects. We also conducted separate analyses for performance IQ (PIQ) and verbal IQ (VIQ), respectively. Finally, we dichotomised the three IQ outcomes and conducted analyses by logistic regression models, using the sample mean minus one SD for the relevant IQ score as described by Eriksen [42].(FSIQ dichotomized at 92.5, PIQ at 88.7, VIQ at 94.0) as a cut-off score for subnormal

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test performance, which implied categorising 209 (15.1%) as subnormal for FSIQ, 232 (16.8%) as subnormal for PIQ, and 229 (16.5%) as subnormal for VIQ.

The study was approved by the DNBC Board of Directors, the DNBC Steering committee, the regional Ethics Committee, the Danish Data Protection Agency, and the CDC Institutional Review Board.

Patient and Public Involvement

Patients were not involved in this study. Results are disseminated to study participants through regular news letters, the website of the DNBC and DNBC on social media.

RESULTS

The children in the LDPS were 60-64 months of age at follow up (mean 5.2 years, range 5.0-5.3 years), and 52% of the children were boys [32]. The mean (standard deviation, std) FSIQ in the study sample was 105.3 (12.8). The study sample for the present analyses, who had information on breastfeeding (n=1385) did not differ from the full LDPS sample with respect to maternal age (mean (std) was 30.9 (4.4) and 30.9 (4.3) respectively), education (median (interquartile range) 13.0 (3.0) in both samples), smoking during pregnancy (31.3% and 31.5%, respectively), and marital status in pregnancy (3.0% and 2.9% married, respectively).

Women who breastfed for less than one month compared to 7-9 and more than 10 months were generally younger, they were more likely to be nulliparous (had not previously given birth), have higher BMI, to have been smokers during pregnancy or to have their children be exposed to tobacco smoke postnatally, have lower IQ, shorter education and lower birthweight of the index child (**table 1**).

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Child IQ at the 5-year examination was directly associated with maternal IQ (Spearman r=0.29, p<0.0001), parental education, birthweight and gestational age, whereas associations were inverse for parity, prepregnancy BMI, smoking during pregnancy and postnatal smoke exposure. Mean IQ was slightly higher for girls compared with boys (data not shown).

Duration of breastfeeding was associated with child IQ at 5 years, even after adjustment for core confounders which included maternal IQ (p from categorical test 0.03) (**table 2**). When analysing duration of breastfeeding as a continuous variable, the association was statistically significant only in the unadjusted analyses (p value for linear trend unadjusted <0.0001, adjusted = 0.68, table 2). Compared to children who were breastfed one month or less, children who were breastfed for 2-3, 4-6, 7-9 and 10 or more months had 3.06 (95% CI 0.39;5.72), 1.90 (95% CI -0.59;4.38), 3.53 (95% CI 1.18;5.87) and 3.28 (95% CI 0.88;5.67) points higher IQ, respectively. These results remained very similar after more extensive adjustment of potential confounders. Furthermore, the estimates for 2-3, 4-6, 7-9, ≥10 months breastfeeding groups were not statistically different from each other (p=0.21), indicating that there was no dose-response, but that the difference in FSIQ lies between those breastfed ≤1 month vs >1 month. Dichotomizing the breastfeeding variable by duration ≤1 month vs >1 month resulted in a 2.98 (95% CI 0.86;5.11) point lower FSIQ in adjusted analysis for children who were breastfed ≤1 month vs >1 month, whereas a dichotomization of breastfeeding by duration ≤6 months vs >6 months resulted in a difference of 1.61 (95% CI 0.29;2.93) points. We subsequently tested for a non-linear association using restricted cubic splines, which provided significantly better fit compared to the linear model (p=0.02), and the model with splines was significantly different from the null (p for non-linearity =0.03).

Adjustment for gestational age and z-score for birth weight in supplementary analyses did not alter our study results, and neither did using a random effect for testing psychologist. Exclusion of mother-child pairs that had never initiated breastfeeding did not change our results for the association between breastfeeding and child IQ, but interestingly the mean maternal IQ in the

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lowest breastfeeding group decreased (from 100.4 to 94.0). When we analysed subscales of IQ, associations were strongest for performance IQ (**table 3**); for verbal IQ effect estimates went in the same direction as for full scale IQ, but were attenuated and not consistently statistically significant across the different adjustment models .

When we stratified for child sex, overall associations were the same and remained statistically significant for both boys and girls, even if confidence intervals widened considerably and effect estimates for the specific breastfeeding groups fluctuated between boys and girls (**supplementary table 1**).

Finally, analyses using dichotomized IQ measures indicated associations in the same direction as the main analyses, but confidence intervals were wide, and associations were generally not statistically significant.

DISCUSSION

In this relatively large study population which was sampled from the Danish National Birth Cohort we investigated associations for duration of breastfeeding with child IQ at age 5 years assessed with the WPPSI-R by a team of trained psychologists. We saw an approximate 3 point difference between those who were breastfed for one month or less compared to those who were breastfed longer.

Our results are in support of the previous studies that have shown beneficial associations for breastfeeding with cognitive measures in children [9-12 43 44]. It furthermore adds to the current body of literature, since we were able to take maternal IQ into account, had detailed information on breastfeeding, clinical assessment of child IQ by a validated tool, as well as a relatively large sample size. Also, we find it relevant that our results are obtained in a population where less than 1% are never breastfed (0.4%), and more than 60% are still breastfed after 6 months, which seems

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to be among the highest breastfeeding rates in the world in a high-income setting [45]. The observed difference in IQ score in our analyses corresponds well with the results of a recent meta-analysis which reported a pooled effect of 3.5 IQ points associated with breastfeeding, and a slightly smaller difference of 2.2 IQ points in studies that were able to adjust for maternal intelligence [3]. According to Jacobson et al the contribution of maternal intelligence to the child's cognitive ability is both genetic and through a more stimulating rearing environment [46]. Furthermore, studies that fail to include maternal intelligence when attempting to show that the link between breastfeeding and cognitive outcome is not attributable to social factors underestimate a critically important factor [22 46]. Our results thus point to some effect of breastfeeding on child IQ over and above that of parent practices and heredity.

We found no clear dose response relation of breastfeeding duration with child cognitive development in our data; rather, our results point to a difference in IQ of more than 3 points between children who are breastfeed for a short period of one month or less compared to those who are breastfed longer. This is in line with the results of a study that used propensity score matching techniques [16] as well as a longitudinal US based study [15]; but stands somewhat in contrast with a meta-analysis from 1999 [8] and a more recent systematic literature review [1] which concluded that cognitive developmental benefits increased with duration of breastfeeding. In our data such a relation was present in the unadjusted analysis only, and we can therefore speculate that effect patterns and sizes may differ between studies depending on other variables that influence IQ, or that previous studies have not investigated such threshold effects of breastfeeding.

In our study sample we categorized the shortest duration as ≤1 month, since very few women reported breastfeeding duration shorter than this, reflecting that by far the majority of mothers in Denmark choose to breastfeed their children. Adding to the difficulty of obtaining an exposure group with shorter duration of breastfeeding is the fact, that women who from the beginning choose

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not to breastfeed may be different from those who do breastfeed; for example, women who rely on medication for various reasons may choose not to breastfeed because of concerns that medication in the breastmilk may harm the infant. This may explain the recent results from the Dutch Generation R Study (N=3761), which compared non-verbal IQ scores among those 'never breastfed' with different durations of breastfeeding, and found a positive association that was, however, attenuated and no longer statistically significant after adjustment for maternal IQ and several potential confounders [24]. Another explanation may be that they assessed non-verbal intelligence by two sub-tests of a Dutch non-verbal IQ test. We used the WPPSI-R, for which reliability coefficients for the IQs for the present age group are very high (0.90-0.96), although they are likely to be somewhat lower when IQs are based on only three verbal and three performance subtests [35].

Our study sample was originally selected to investigate effects of low to moderate alcohol intake on child development, however, this did not seem to inflict on breastfeeding behaviour, since rates of breastfeeding in our sample were similar to rates in the DNBC overall. The differences between women who breastfeed or not can only be addressed entirely in a randomized controlled trial, such as the large PROBIT-study in Belarus, where randomization of clinics and hospitals to breastfeeding promotion resulted in a substantial increase both in breastfeeding exclusivity and duration of any breastfeeding. At age 6.5 years children from the PROBIT-study were followed up (n=13889), and children from the intervention arm scored 7.5 points higher on verbal IQ from the Wechsler Abbreviated Scales of Intelligence, providing strong evidence for a beneficial effect of prolonged breastfeeding on children's cognitive development [5]. Such effects may have a long term impact, as suggested by a Danish observational study, which showed a beneficial association between duration of breastfeeding and adult intelligence in two non-overlapping samples (n=973 men and women, n=2280 men, respectively) [18]. According to Huang et al subsequent schooling and other socialization experiences during adolescence do not eliminate the breastfeeding gap that appears in very early childhood [47]. Furthermore, recent studies have found that the effects of

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breastfeeding may translate into substantial educational differences in adult life [20] and economic gains to society [48].

Previous studies have suggested three different mechanisms underlying such associations: differences in nutrient compositions between human milk and formula, interplay around the feeding situation, and unidentified factors that correlate with both breastfeeding and neurodevelopment, including residual confounding [18]. With respect to differences in nutrient content, long chained polyunsaturated n-3 fatty acids, and docosahexaenoic acid (DHA) in particular, may be involved. DHA is deemed an essential fatty acid, because it cannot be intrinsically produced and must hence be provided by the diet, with fatty fish as the main source. Results from the Danish National Birth Cohort showed both breastfeeding and maternal fish intake to be independently associated with developmental milestones in the young offspring [49].

It is a main strength of our study that we were able to include the most important confounders: maternal intelligence, parental education and home environment, which in separate analyses have been shown to account for 19-29% of the variance in IQ [50 51], also in analyses conducted within the LDPS [52]. Additional strengths to our study include the relatively large number of mother-children pairs in our sample, and the fact that child IQ was assessed by a state of the art clinical intelligence test with high reliability coefficients for IQ for the present age group.

The main limitations of our study are the different possible sources of bias inherent in an observational study design. As is the case in most large cohort studies, participants in the DNBC were generally better off and more health conscious compared to the overall Danish population of pregnant women. However, according to a study by Nøhr et al this selection did not result in biased estimates for selected aetiological associations [53]. Furthermore, at the time the women in the DNBC reported on breastfeeding duration they were not aware of the outcome under study, making it less likely that their reporting was affected by this. Still it may be regarded a weakness, that

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breastfeeding was reported at two occasions, when children were 6 and 18 months old, requiring mothers to recall their practices used a few months before the actual assessment. Also, we were not able to investigate dose of breastfeeding, since we did not restrict the exposure to exclusive breastfeeding.

The LDPS oversampled mothers with moderate alcohol intake in pregnancy, which we adjusted for by weighing all analyses by the relevant sampling fractions. Furthermore, maternal alcohol use was not strongly associated with poorer performance at 5 years [32 54 55]. We therefore believe it to be unlikely that any of the associations we observe are due to selection bias caused by the sampling in the LDPS. However, it may still be regarded as a limitation, that participants for this sub-study of the DNBC were not selected at random, but by very specific exposure criteria related to maternal behavior during pregnancy. We believe that this has been remedied by our weighing of all analyses by sampling fractions, and that our results can therefore be seen as generalizable to the Danish National Birth Cohort, and the Danish population.

Associations tended to be attenuated with increasing number of covariates in our analyses, suggesting unadjusted confounding or sparse data bias [56]. As is always the case in observational studies, we cannot exclude that our results are affected by residual confounding or confounding by factors not accounted for in our analyses. Furthermore, there may be postnatal factors influencing child IQ that we have not been able to take into consideration; however, these may not act as true confounders but rather as intermediates, in which case they should not be included in the analyses. Current recommendations from the World Health Organization are that women should breastfeed exclusively for the first six months. Our finding of a 3 point difference in IQ associated with any duration of breastfeeding longer than one month is in support of current recommendations, and is even a relaxed message to mothers who struggle with exclusive breastfeeding. Although negligible when considered at the individual level, seen from a public health perspective a difference of 3 IQ points must be considered substantial, and smaller effects have previously led to quite conservative

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precautionary recommendations, for example with respect to adverse effects of maternal exposure to environmental toxicants [57].

In conclusion, in this large sample with high quality assessment of child IQ, we found support of a beneficial association for breastfeeding with child IQ at 5 years of age, while adjusting for maternal IQ and parental education, which only few previous studies have been able to do. Taking the necessary precautions that our results may reflect residual confounding, our findings support current recommendations with respect to breastfeeding in relation to cognitive development of the child.

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Author's contributions

The authors' responsibilities were as follows:

SFO and MS designed research; ELM, UK, SFO, TIH, JO and MS provided essential materials and conducted research; MS and TIH performed statistical analysis; MS, TIH, ELM, UK, SFO interpreted the results. MS wrote paper; MS had primary responsibility for final content. All authors read and approved the final manuscript. All authors had full access to study data. None of the authors had a conflict of interest.

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Figure legend

Figure 1
Overview of the study sample from the Lifestyle During Pregnancy Study

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Table 1: Study participants in the Lifestyle During Pregnancy Study distributed by maternal and child characteristics and by breastfeeding duration (n=1385)

		Breastfeeding duration					
	Overall	**≤1 month**	**2-3 months**	**4-6 months**	**7-9 months**	**≥10 months**	**p[1]**
Maternal age at pregnancy (years)[2]	30.9 (4.4)	30.9 (4.5)	29.9 (4.4)	30.1 (4.2)	30.9 (4.1)	32.0 (4.3)	<0.0001
Parity[2]							0.0003
Nulliparous	676 (48.8)	80 (57.6)	82 (56.2)	122 (49.0)	194 (49.0)	198 (43.5)	
1 child	467 (33.7)	41 (29.5)	50 (34.3)	88 (35.3)	142 (35.9)	146 (32.1)	
≥2 children	242 (17.5)	18 (13.0)	14 (9.6)	39 (15.7)	60 (15.2)	111 (24.4)	
Prepregnancy BMI (kg/m²)[2]	22.6 (4.5)	22.8 (4.1)	23.3 (4.7)	22.7 (4.7)	22.6 (4.0)	22.2 (4.3)	<0.0001
Marital status[2]							0.3
Single	37 (2.7)	4 (2.9)	2 (1.4)	11 (4.4)	7 (1.8)	13 (2.9)	
Cohabiting with partner	1348 (97.3)	135 (97.1)	144 (98.6)	238 (95.6)	389 (98.2)	442 (97.1)	
Alcohol, drinks/wk in pregnancy[2]	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.3
Smoking in pregnancy[2]							<0.0001
Yes	444 (32.1)	54 (38.9)	66 (45.2)	91 (36.6)	102 (25.8)	131 (28.8)	
No	941 (67.9)	85 (61.2)	80 (54.8)	158 (63.5)	294 (74.2)	324 (71.2)	
Postnatal smoking[2]							<0.0001
Yes	449 (32.4)	51 (36.7)	74 (50.7)	97 (39.0)	98 (24.8)	129 (28.4)	
No	936 (67.6)	88 (63.3)	72 (49.3)	152 (61.0)	298 (75.3)	326 (71.7)	
Maternal IQ [2]	100.0 (14.9)	100.4 (14.9)	94.3 (14.8)	97.1 (13.8)	102.2 (14.0)	103.3 (14.9)	<0.0001
Parental education (years)[2]	13.0 (3.0)	13.0 (3.5)	12.3 (3.0)	12.5 (2.5)	13.0 (2.5)	13.5 (2.5)	<0.0001
Child sex[2]							0.5
Girls	654 (47.2)	74 (53.2)	66 (45.2)	115 (46.2)	178 (45.0)	221 (48.6)	
Boys	731 (52.8)	65 (46.8)	80 (54.8)	134 (53.8)	218 (55.1)	234 (51.4)	
Gestational age at birth (weeks)[2]	40.1 (1.5)	40.1 (1.7)	39.8 (1.7)	40.0 (1.5)	40.2 (1.5)	40.1 (1.4)	0.3
Birth weight *z*-score[2]	0.00 (0.97)	0.02 (0.98))	-0.22 (0.93)	0.01 (0.95)	-0.01 (0.90)	0.11 (1.00)	0.005

1 p-value from chi² test (categorical variables: parity, marital status, smoking in pregnancy, postnatal smoking, child sex), F-test one way anova (normally distributed variables: maternal age, maternal IQ, gestational age, *z*-score for birth weight), or Kruskal-Wallis test (non-normally distributed variables: prepregnancy BMI, alcoholic drinks/week in pregnancy, parental education)

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2 Values are mean (SD) (for normally distributed variables: maternal age, maternal IQ, gestational age, *z*-score for birth weight), median (IQR) (for non-normally distributed variables: prepregnancy BMI, alcoholic drinks/week in pregnancy, parental education) or n (%) (for categorical variables: parity, marital status, smoking in pregnancy, postnatal smoking, child sex)

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Table 2: Full scale IQ among 1385 children from the Lifestyle During Pregnancy Study in association with breastfeeding duration

		Crude		Adjusted for core confounders[2]		Adjusted for all potential confounders[2]	
Breastfeeding	Mean score Full scale IQ[1]	Mean difference	95% CI	Mean difference	95% CI	Mean difference	95% CI
	101.93						
Per month breastfeeding		0.33	(0.19; 0.47)	0.04	(-0.10; 0.18)	0.07	(-0.08;0.21)
		<0.0001[3]		*0.58*[3]		*0.37*[3]	
<=1 month	98.84	Ref.	-	Ref.	-	Ref.	-
2-3 months		3.92	(1.13;6.71)	3.06	(0.39;5.72)	3.24	(0.61;5.86)
4-6 months		4.37	(1.87;6.87)	2.03	(-0.38;4.44)	1.70	(-0.69;4.08)
7-9 months		6.61	(4.21;9.01)	3.53	(1.18;5.87)	3.36	(1.06;5.66)
≥10 months		7.85	(5.46;10.25)	3.28	(0.88;5.67)	3.41	(1.02;5.80)
		<0.0001[4]		*0.03*[4]		*0.02*[4]	

1. Wechsler Preschool and Primary Scale of Intelligence-Revised
2. Covariate models; core confounders: Maternal IQ, parental education, smoking during pregnancy, child's age at testing, testing psychologist. All potential confounders: core + maternal age at pregnancy, maternal marital status, maternal parity, sex of child, maternal prepregnant BMI, postnatal parental smoking, health index, home environment, maternal average alcohol intake during pregnancy.
3. *P*-value for the hypothesis of no trend in IQ-scores across levels of exposure
4. *P*-value for the hypothesis of no difference in IQ-scores across levels of exposure, categorical test

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Table 3: Verbal and Performance scale IQ among 1385 children from the Lifestyle During Pregnancy Study in association with breastfeeding duration

Verbal IQ		Crude		Adjusted for core confounders[2]		Adjusted for all potential confounders[2]	
	Mean score Verbal IQ[1]	Mean difference	95% CI	Mean difference	95% CI	Mean difference	95% CI
	104.09						
Per month breastfeeding		0.20	(0.08; 0.32)	-0.003	(-0.12; 0.12)	0.06	(-0.09;0.22)
		0.001[3]		*0.95*[3]		*0.41*[3]	
<=1 month	100.61	Ref.	-	Ref.	-	Ref.	-
2-3 months		1.77	(-0.61;4.15)	0.88	(-1.41;3.18)	1.04	(-1.24;3.33)
4-6 months		2.79	(0.66;4.92)	1.12	(-0.96;3.20)	1.07	(-1.01;3.15)
7-9 months		4.42	(2.37;6.47)	2.33	(0.30;4.35)	2.19	(0.17;4.20)
≥10 months		4.95	(2.91;6.99)	1.71	(-0.36;3.77)	2.17	(0.08;4.26)
		<0.0001[4]		*0.18*[4]		*0.18*[4]	
Performance IQ		Crude		Adjusted for core confounders[2]		Adjusted for all potential confounders[2]	
	Mean score Perform. IQ[1]	Mean difference	95% CI	Mean difference	95% CI	Mean difference	95% CI
	103.64						
Per month breastfeeding		0.39	(0.22; 0.57)	0.06	(-0.12; 0.24)	0.09	(-0.09;0.27)
		<0.0001[3]		*0.51*[3]		*0.31*[3]	
<=1 month	97.08	Ref.	-	Ref.	-	Ref.	-
2-3 months		5.49	(1.96;9.03)	4.95	(1.55;8.36)	5.30	(1.96;8.63)
4-6 months		5.13	(1.73;8.30)	2.39	(-0.71;5.48)	2.13	(-0.90;5.17)
7-9 months		7.52	(4.48;10.57)	3.84	(0.83;6.84)	3.87	(0.93;6.81)
≥10 months		9.29	(6.26;12.32)	4.06	(0.99;7.13)	4.34	(1.29;7.39)
		<0.0001[4]		*0.03*[4]		*0.009*[4]	

1. Wechsler Preschool and Primary Scale of Intelligence-Revised, verbal and performance scale, respectively

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2. Covariate models; core confounders: Maternal IQ, parental education, smoking during pregnancy, child's age at testing, testing psychologist. All potential confounders: core + maternal age at pregnancy, maternal marital status, maternal parity, sex of child, maternal prepregnant BMI, postnatal parental smoking, health index, home environment, maternal average alcohol intake during pregnancy.
3. *P*-value for the hypothesis of no trend in IQ-scores across levels of exposure
4. *P*-value for the hypothesis of no difference in IQ-scores across levels of exposure, categorical test

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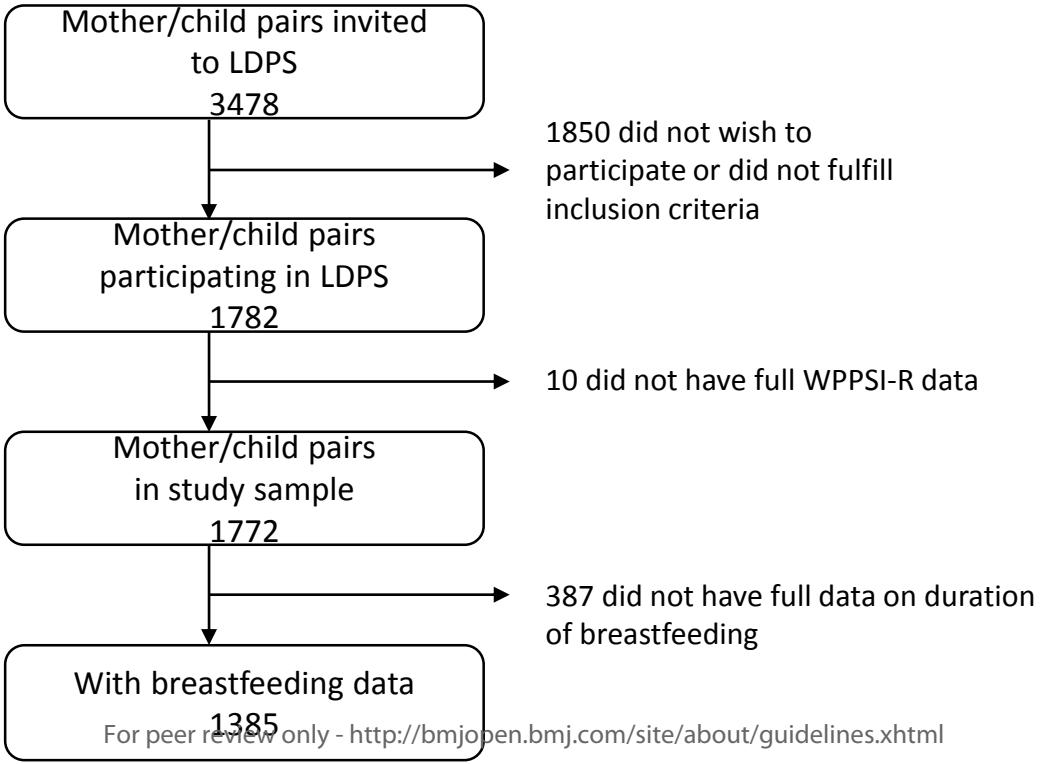

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Supplementary table 1: Full scale IQ among 1385 children from the Lifestyle During Pregnancy Study in association with breastfeeding duration, stratified by child sex

Girls		Crude		Adjusted for core confounders		Adjusted for all potential confounders[2]	
	Mean score Full IQ[1]	Mean difference	95% CI	Mean difference	95% CI	Mean difference	95% CI
	106.77						
Per month breastfeeding		0.32	(0.11; 0.52)	0.07	(-0.14; 0.27)	-0.04	(-0.26;0.17)
		0.003[3]		*0.52*[3]		*0.70*[3]	
<=1 month	101.89	Ref.	-	Ref.	-	Ref.	-
2-3 months		7.56	(3.34;11.79)	7.10	(3.19;11.01)	6.99	(3.01;10.97)
4-6 months		2.17	(-1.44;5.79)	-0.73	(-4.09;2.63)	-1.12	(-4.58;2.34)
7-9 months		4.30	(0.74;7.86)	-0.25	(-3.64;3.15)	-1.18	(-4.63;2.27)
≥10 months		8.09	(4.59;11.59)	3.25	(-0.10;6.61)	2.01	(-1.45;5.46)
		<0.0001[4]		*<0.0001*[4]		*<0.0001*[4]	
Boys		Crude		Adjusted for core confounders		Adjusted for all potential confounders[2]	
	Mean score Full IQ[1]	Mean difference	95% CI	Mean difference	95% CI	Mean difference	95% CI
	102.44						
Per month breastfeeding		0.33	(0.15; 0.52)	0.03	(-0.16; 0.22)	0.06	(-0.13;0.25)
		0.0004[3]		*0.75*[3]		*0.52*[3]	
<=1 month	96.53	Ref.	-	Ref.	-	Ref.	-
2-3 months		1.98	(-1.61;5.56)	0.33	(-3.16;3.83)	1.32	(-2.11;4.75)
4-6 months		5.85	(2.52;9.17)	2.87	(-0.41;6.16)	3.28	(0.08;6.48)
7-9 months		8.39	(5.26;11.52)	5.36	(2.27;8.45)	4.98	(1.96;8.00)
≥10 months		7.30	(4.15;10.45)	2.13	(-1.09;5.36)	3.00	(-0.20;6.21)
		<0.0001[4]		*0.0005*[4]		*0.009*[4]	

1. Wechsler Preschool and Primary Scale of Intelligence-Revised

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2. Covariate models; core confounders: Maternal IQ, parental education, smoking during pregnancy, child's age at testing, testing psychologist. All potential confounders: core + maternal age at pregnancy, maternal marital status, maternal parity, sex of child, maternal prepregnant BMI, postnatal parental smoking, health index, home environment, maternal average alcohol intake during pregnancy.
3. *P*-value for the hypothesis of no trend in IQ-scores across levels of exposure
4. *P*-value for the hypothesis of no difference in IQ-scores across levels of exposure, categorical test

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STROBE Statement—Checklist of items that should be included in reports of ***cohort 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 X – p. 4
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found X – p. 4-5
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported X – p. 6-7
Objectives	3	State specific objectives, including any prespecified hypotheses X – p. 7
Methods		
Study design	4	Present key elements of study design early in the paper X – p. 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection X – p. 7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up X – p. 7-8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable X – p. 8-10
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 X – p. 7-10
Bias	9	Describe any efforts to address potential sources of bias X – p. 9-12
Study size	10	Explain how the study size was arrived at X – p. 9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why X – p. 8-10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding X – p. 10-12
		(*b*) Describe any methods used to examine subgroups and interactions X – p. 12
		(*c*) Explain how missing data were addressed X – p. 12
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses X – p. 12
Results		

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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 X – p. 9
		(b) Give reasons for non-participation at each stage X – p. 9
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders X – p. 12
		(b) Indicate number of participants with missing data for each variable of interest X – p. 11
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time X – p. 12-13
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 X – p. 13-14, Table 2
		(*b*) Report category boundaries when continuous variables were categorized X – Table 1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses X – p. 13-14
Discussion		
Key results	18	Summarise key results with reference to study objectives X – p. 14
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 X – p. 15,18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence X – p. 15-18
Generalisability	21	Discuss the generalisability (external validity) of the study results X – p. 15-16
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 X – p. 1-2

*Give information separately for exposed and unexposed groups.

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 http://www.strobe-statement.org.

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Is breastfeeding associated with offspring IQ at age 5? Findings from prospective cohort: lifestyle during pregnancy study

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Is breastfeeding associated with offspring IQ at age 5? Findings from prospective cohort: lifestyle during pregnancy study.

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ABSTRACT

Objectives

Breastfeeding is associated with health benefits for both mother and child, but many studies focusing on neurodevelopment have lacked information on important confounders and few randomized trials exist. Our objective was to examine the influence of breastfeeding on child IQ at 5 years of age while taking maternal IQ and other relevant factors into account.

Design, setting, participants and outcome measures

We used data from The Lifestyle During Pregnancy Study, consisting of 1782 mother-child pairs sampled from the Danish National Birth Cohort (n=101,042). Child IQ was assessed at age 5 years by the Wechsler Primary and Preschool Scales of Intelligence-Revised. On the same occasion maternal intelligence was assessed by Wechsler Adult Intelligence Scale and Raven's Standard Progressive Matrices. Exposure data on duration of breastfeeding (n=1385) were extracted from telephone interviews conducted when the child was 6 and 18 months, and analyses were weighted by relevant sampling fractions.

Results

In multivariable linear regression analyses adjusted for potential confounders breastfeeding was associated with child IQ at 5 years (categorical chi^2 test for overall association p=0.03). Compared to children who were breastfed ≤1 month, children breastfed for 2-3, 4-6, 7-9 and 10 or more months had 3.06 (95% CI 0.39;5.72), 1.90 (95% CI -0.59;4.38), 3.53 (95% CI 1.18;5.87) and 3.28 (95% CI 0.88;5.67) points higher IQ after adjustment for core confounders, respectively. There was no dose-response relation and further analyses indicated that the main difference in IQ was between breastfeeding ≤1 month vs >1 month.

Conclusions

Breastfeeding duration of 1 month or shorter compared to longer periods was associated with approximately 3 points lower IQ, but there was no evidence of a dose-response relation in this

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prospective birth cohort, where we were able to adjust for some of the most critical confounders, including maternal intelligence.

Article Summary

- Study based on a large population based pregnancy cohort with prospective data assessments
- This study of the association between breastfeeding and child IQ took into account maternal intelligence and home environment, two critical confounders
- Very few women did not breastfeed (6/1385, 0.4%), so it was not possible to investigate this group separately
- Postnatal factors may have influenced child IQ that we have not been able to take into consideration

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Competing Interests

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All authors declare no competing interests

Data sharing statement

Data are not available for this study due to the data regulations from the relevant cohort responsible: the Lifestyle During Pregnancy Study and the Danish National Birth Cohort.

Abbreviations

BMI: body mass index

CI: confidence interval

DNBC: Danish National Birth Cohort

FSIQ: Full Scale IQ

IQ: intelligence quotient

IQR: Interquartile range

LDPS: Lifestyle During Pregnancy Study

SD: standard deviation

WPPSI-R: Wechsler Primary and Preschool Scales of Intelligence-Revised

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INTRODUCTION

There is firm evidence for the beneficial effects of breastfeeding on a wide range of maternal and child outcomes [1 2] and to ensure healthy growth and development the World Health Organization recommends that all children are exclusively breastfed for the first six months of life, followed by partial breastfeeding [3].

Since the very early observations by Hoefer and Hardy in 1929 of a positive association for breastfeeding with cognitive performance at age 7-13 years [4], a large body of evidence has provided results in this field. Due to difficulties and ethical concerns intrinsic in designing an intervention targeting breastfeeding duration, few randomized trials exist [5-7]. These have shown beneficial effects of breastfeeding in relation with child neurodevelopmental outcomes, and one of these indicated that exclusive breastfeeding may not confer superior effects compared to breastfeeding and complementary foods introduced at 4 months of age [7]. Several observational studies have shown positive associations for duration of breastfeeding with cognitive developmental benefits in childhood [8-16] and in adult life [17-20]. Differences in nutrient compositions between human milk and formula have been suggested as one of several potential mechanisms underlying a beneficial effect of breastfeeding on cognitive development [18]. In some studies, crude positive associations between breastfeeding and cognitive endpoints were attenuated upon adjustment for potential confounders, including socioeconomic background [21-25], which has made authors conclude that breastfeeding may be a proxy for home environment or parental practices rather than a causal factor in itself [21-27]. However, many studies have been relatively small (n<500), have had limited details on breastfeeding or have not been able to take into account some of the critical confounders including maternal intelligence [9 12 26]. A systematic review and meta-analysis in 2013 concluded that breastfeeding is associated with increased performance in intelligence tests in childhood and adolescence of 3.5 points on average, and that maternal IQ, despite being an important confounder, can account only for part of this association [3]. While the practical implications of such moderate

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differences in intelligence tests can be questioned, a recent study from Brazil [20] indicated that a cognitive advantage by breastfeeding was maintained until adulthood mediating effects on life outcomes including educational attainment and income even in a setting without strong social patterning of breastfeeding [20 28].

The aim of the present study was to assess, in an observational setting, the influence of breastfeeding on child IQ at 5 years of age while taking maternal IQ and home environment into account in the Lifestyle During Pregnancy Study.

SUBJECTS AND METHODS

The aim of the Lifestyle During Pregnancy Study (LDPS) is to examine the relation between maternal lifestyle during pregnancy and offspring neurodevelopment by the age of 5 years [29]. The study is a subsample of the large prospective Danish National Birth Cohort (DNBC) [30]. The DNBC recruited >100,000 pregnant women between 1996 and 2002, and data collection instruments included two pregnancy telephone interviews (in gestational weeks 12 and 30 approximately), two telephone interviews postpartum (at 6 and 18 months postpartum), as well as a detailed food frequency questionnaire (FFQ) which was mailed to the women in gestational week 25 and covered the dietary intake in the previous month [31]. Information on birth outcome was obtained from the Medical Birth Registry.

The LPDS consists of 3478 mother-child dyads selected from the DNBC with oversampling of pregnant women with moderate weekly alcohol intake, alcohol binge drinkers and women with high vs. low fish intake, iron intake and duration of breastfeeding, respectively [29]. A detailed description of the LDPS, including the sampling frame, has been published elsewhere [29 32]. In brief, 3478 mother-child pairs from the DNBC were invited to participate in a follow up around the time when the children turned 5 years. Of the selected dyads 1782 mothers and children (51%) took part in a comprehensive three-hour assessment of neurodevelopment focusing on global cognition, specific

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cognitive functions, and behaviour of the child, and additionally mothers completed tests of adult intelligence (IQ) [29].

Exclusion criteria for the follow-up in LDPS were multiple pregnancies, inability to speak Danish, impaired hearing or vision of the child likely to compromise the ability to perform the cognitive tests, and congenital disorders likely to cause mental retardation, including trisomy 21 [29]. All test procedures were standardised in detail and carried out by 10 trained psychologists. The examinations took place in the four largest cities of Denmark: Copenhagen, Aarhus, Odense and Ålborg [29].

Exposure variables

At both telephone interviews postpartum, when the child was 6 and 18 months, respectively, mothers answered questions about breastfeeding. At the 6 months' interview they reported whether they had never breastfed, were currently breastfeeding or had stopped breastfeeding. Those who were no longer breastfeeding reported the child's age when daily breastfeeding stopped. At 18 months postpartum mothers were asked whether they had breastfed their child beyond 6 months, and if so, whether they were still breastfeeding; if not, they reported the child's age at discontinuation of breastfeeding. Combining the answers from these two data collection instruments we constructed a measure of duration of any breastfeeding, not distinguishing between exclusive and supplemented breastfeeding.

Outcome variables

We used child IQ assessed at the follow up examination at age 5 with the Wechsler Primary and Preschool Scales of Intelligence-Revised (WPPSI-R) [33]. The WPPSI-R is one of the most widely used standardized tests of intelligence for children of 2-7 years of age. In its full form the test battery for WPPSI-R comprises 5 verbal subtests and 5 performance (non-verbal) subtests from

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which verbal IQ, performance IQ, and full scale (FS) IQs are derived. The length of the examination was accommodated to match the age of the study participants (5 years), and therefore a short form of the WPPSI-R was used, which included three verbal (Arithmetic, Information and Vocabulary) and three performance subtests (Block Design, Geometric Design and Object Assembly). Standard procedures were used to prorate IQs from the shortened forms of the tests [34], and since no Danish WPPSI-R norms were available at the time of the study Swedish norms were used to derive scaled scores and IQs [35]. The use of Swedish norms might cause a slight shift in the IQ distribution, and the theoretical mean of 100 (SD 15) cannot necessarily be expected in this sample. However, as the analyses conducted in this study are internal comparisons across exposure, a systematic shift in the IQ distribution should not affect our results.

Mother-child pairs available for analysis

Out of the total number of participants in the LDPS (n=1782), the study sample for these analyses consisted of those 1772 (99.4%) mother-child dyads in the LDPS with full outcome data on the WPPSI-R. Full information on breastfeeding was available for 1385 (78.2%) of these as lined out in **figure 1**.

Covariates

We used information from the prenatal telephone interviews, a questionnaire at the 5-year follow-up examination, and from the Medical Birth Registry and Central Person Registry to define potential confounders that we included in our analyses as covariates. Maternal IQ was assessed at the follow-up examination by two verbal subtests (information and vocabulary) from the Wechsler Adult Intelligence Scale [36] and non-verbal IQ by the Raven's Standard Progressive Matrices [37]. Raw scores of each of the two subtests (information and vocabulary) were standardised based on the results from the full sample. Subsequently scores from each of the two subtests were weighted

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equally into one combined score, and this combined score was then restandardised to a scale with a mean of 100 and SD of 15.

We defined the covariates as follows: parity (0, 1, ≥2 previous births); prenatal maternal smoking (yes, no); maternal pre-pregnancy body mass index (BMI) (weight(kg)/height(m)^2); parental education (average educational length in years for the parents or of mother if that of the father was unavailable); marital status (single during pregnancy or at 5 year follow up, married/cohabitating at both); postnatal parental smoking (yes if mother or father smoked in the home, no if otherwise); maternal average alcohol intake in pregnancy (0, 1-4, 5-8, ≥9 drinks/week); an index of the child's health status (dichotomized as normal or suboptimal in the presence of any illness, diseases, handicaps and/or medication with potential influence on test performance); an index of the quality of postnatal home environment (dichotomized as normal or suboptimal in the presence of two or more of the following seven conditions: not living with a biological parent, changes in caregiver, day care for more than 8 hours/day before age 3, 14+ days of separation from the parents, breakfast irregularity, maternal depression and parental alcohol use above the maximum recommended level by the Danish Health Authority at the time of follow-up of 14 (women) or 21 (men) drinks/week); maternal age at pregnancy (in years); maternal IQ (score), sex of child (girl, boy) and age of child at the follow-up examination. Gestational age was calculated from the last menstrual period provided by the mother at study recruitment (gestation week 6-10) or from the expected date of delivery provided by the woman during the second telephone interview (gestation week 30), which was most often based on ultrasound results [38]. The date of birth was extracted from the Central Person Registry, and the midwife who attended the child's birth recorded birth weight which we extracted from the Medical Birth Registry and calculated sex-specific weight *z*-score at birth on the basis of published reference data [39 40].

The 10 trained psychologists who carried out the testing of the study participants were blinded to the child's exposure status, and we took tester differences into account by the inclusion of a

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categorical variable with 10 levels in the statistical analyses. In a sensitivity analysis tester was taken into consideration in the analysis by a random effect.

Statistical analyses

We used the median (interquartile range (IQR)) to describe skewed variables; mean (SD) for normally distributed variables; and n/percentage for categorical variables. Throughout, when analyzing associations with continuous measures *p*-values from F tests (type II) are presented, for associations with categorical measures *p*-values from chi^2 test (type III) are presented.

The exposure variable, breastfeeding duration, was examined in relation to child IQ both as a continuous variable (breastfeeding duration in months), and categorized as any breastfeeding ≤1 month, 2-3 months, 4-6 months, 7-9 months or ≥10 months. When analyzing breastfeeding as a continuous measure, we present p-values from F test of trend across months of breastfeeding, when looking at breastfeeding as a categorical variable we present p-values from overall F test of any difference in IQ score across groups of breastfeeding duration.

Furthermore, for supplementary analysis we dichotomized the exposure variable in order to examine the difference in FSIQ according to duration of any breastfeeding ≤1 month vs. >1 month, and ≤6 months vs. >6 months.

We present differences in WPPSI-R FSIQ scores across exposure levels by using multivariable linear regression models to obtain β estimates and corresponding 95% confidence intervals (CIs). In our multivariable analyses we adjusted for potential confounders in two steps: maternal IQ, parental education, maternal smoking during pregnancy, age of child at the follow-up examination and tester were considered core confounders, and we considered this our main analysis.; in a more extensively adjusted model we furthermore included the following potential confounders: maternal age, maternal marital status, parity, sex of child, maternal prepregnancy BMI, postnatal parental smoking, health index, home environment and maternal average alcohol intake during pregnancy.

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Supplementary to this, a non-linear relation between duration of breastfeeding (continuous) and child FSIQ was tested comparing a linear model to a model using restricted cubic splines with 3 knots placed at the 25th, 50th and 75th percentiles [41]. To test for deviations from linearity, we used a likelihood ratio test (P curvature, F test) to compare the linear model with a model fit that based based on restricted cubic splines.

Due to the oversampling of study participants according to certain behavioural exposures, as described above (moderate alcohol use, binge drinking, fish, iron intake and duration of breastfeeding) all analyses were weighted by sampling fractions to account for the complex stratified sampling design [32].

For the 1385 mother-child pairs with data on exposures and outcome, data on child's age at testing and testing psychologist was complete, whereas information on other covariates was missing for approximately 8% of participants (ranging from 7.9% for maternal smoking during pregnancy to 9.5% for maternal prepregnancy BMI). We substituted missing covariate values by multiple imputation using the standard procedure PROC MI in SAS, which utilizes all the information available and imputes 5 different datasets based on which we calculated 5 different sets of effect and variance estimates, and subsequently used the procedure PROC MIANALYZE to compute a combined estimate and standard error for each regression coefficient in our analyses. Results from alternative analytic strategies using complete case and substitution of missing values with mean/mode imputation did not alter our conclusions.

All analyses were carried out using SAS statistical software (version 9.4, SAS Institute, Cary, North Carolina, USA), and all tests were two-sided with statistical significance set to $p<0.05$.

Supplementary analyses

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To avoid overadjustment gestational age and birth weight *z*-score were not included in our main models. As supplementary analysis we furthermore excluded those mother-child pairs who never initiated breastfeeding (n=6). We stratified by child sex, in order to examine potential sex specific effects. We also conducted separate analyses for performance IQ (PIQ) and verbal IQ (VIQ), respectively. Finally, we dichotomised the three IQ outcomes and conducted analyses by logistic regression models presenting chi^2 tests evaluating statistical significance for overall association, using the sample mean minus one SD for the relevant IQ score as described by Eriksen [42](FSIQ dichotomized at 92.5, PIQ at 88.7, VIQ at 94.0) as a cut-off score for subnormal test performance, which implied categorising 209 (15.1%) as subnormal for FSIQ, 232 (16.8%) as subnormal for PIQ, and 229 (16.5%) as subnormal for VIQ.

The study was approved by the DNBC Board of Directors, the DNBC Steering committee, the regional Ethics Committee, the Danish Data Protection Agency, and the CDC Institutional Review Board.

Patient and Public Involvement

Patients were not involved in this study. Results are disseminated to study participants through regular news letters, the website of the DNBC and DNBC on social media.

RESULTS

The children in the LDPS were 60-64 months of age at follow up (mean 5.2 years, range 5.0-5.3 years), and 53% of the children were boys [32]. The mean (standard deviation, std) FSIQ in the study sample was 105.3 (12.8). The study sample for the present analyses, who had information on breastfeeding (n=1385) did not differ from the full LDPS sample with respect to maternal age (mean (std) was 30.9 (4.4) and 30.9 (4.3) respectively), education (median (interquartile range) 13.0

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(3.0) in both samples), smoking during pregnancy (32.4% and 31.5%, respectively), and cohabitation status in pregnancy (2.7% and 2.9% single, respectively).

Women who breastfed for less than one month compared to 7-9 and more than 10 months were generally younger, they were more likely to be nulliparous (had not previously given birth), have higher BMI, to have been smokers during pregnancy or to have their children be exposed to tobacco smoke postnatally, and have lower IQ (**table 1**). Duration of parental education and birthweight *z*-score was highest for children breastfed for 10 months or longer, and lowest for those who were breastfed for 2-3 months.

Child IQ at the 5-year examination was directly associated with maternal IQ (Spearman r=0.29, p<0.0001), parental education, birthweight *z*-score and gestational age, whereas associations were inverse for parity, prepregnancy BMI, smoking during pregnancy and postnatal smoke exposure (data not shown). Mean IQ was slightly higher for girls compared with boys (supplementary table 1).

Duration of breastfeeding was associated with child IQ at 5 years, even after adjustment for core confounders which included maternal IQ (p from overall categorical test 0.03) (**table 2**). Compared to children who were breastfed one month or less, children who were breastfed for 2-3, 4-6, 7-9 and 10 or more months had 3.06 (95% CI 0.39;5.72), 2.03 (95% CI -0.38;4.44), 3.53 (95% CI 1.18;5.87) and 3.28 (95% CI 0.88;5.67) points higher IQ, respectively. These results remained very similar after more extensive adjustment of potential confounders. When analysing duration of breastfeeding as a continuous variable, the association was statistically significant only in the unadjusted analyses (unadjusted β (95% CI): 0.33 (0.19;0.47), adjusted β: 0.04 (-0.10;0.18)). Furthermore, the estimates for 2-3, 4-6, 7-9, ≥10 months breastfeeding groups were not statistically different from each other (F-test categorical test for overall association omitting mother-child pairs with breastfeeding <= 1 month, p=0.21), indicating that there was no dose-response, but that the difference in FSIQ lies between those breastfed ≤ 1 month vs >1 month. Dichotomizing the

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breastfeeding variable by duration ≤ 1 month vs >1 month resulted in a 2.98 (95% CI 0.86;5.11) point lower FSIQ in adjusted analysis for children who were breastfed ≤1 month vs >1 month, whereas a dichotomization of breastfeeding by duration ≤6 months vs >6 months resulted in a difference of 1.61 (95% CI 0.29;2.93) points. We subsequently tested for a non-linear association using restricted cubic splines, which provided significantly better fit compared to the linear model (p=0.02), and the model with splines was significantly different from the null (p for non-linearity =0.03).

Adjustment for gestational age and z-score for birth weight in supplementary analyses did not alter our study results, and neither did using a random effect for testing psychologist. Exclusion of mother-child pairs that had never initiated breastfeeding (6/1385, 0.4%) did not change our results for the association between breastfeeding and child IQ, but interestingly the mean maternal IQ in the lowest breastfeeding group decreased (from 100.4 to 94.0). When we analysed subscales of IQ, associations were strongest for performance IQ (**table 3**); for verbal IQ effect estimates went in the same direction as for full scale IQ, but were attenuated and not consistently statistically significant across the different adjustment models .

When we stratified for child sex, overall associations were the same and remained statistically significant for both boys and girls, even if confidence intervals widened considerably and effect estimates for the specific breastfeeding groups fluctuated between boys and girls (**supplementary table 1**).

Finally, analyses using dichotomized IQ measures indicated associations in the same direction as the main analyses, but confidence intervals were wide, and associations were generally not statistically significant.

DISCUSSION

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In this relatively large study population which was sampled from the Danish National Birth Cohort we investigated associations for duration of breastfeeding with child IQ at age 5 years assessed with the WPPSI-R by a team of trained psychologists. We saw an approximate 3-point difference in IQ between those who were breastfed for one month or less compared to those who were breastfed longer.

Our results are in support of the previous studies that have shown beneficial associations for breastfeeding with cognitive measures in children [9-12 43 44]. It furthermore adds to the current body of literature, since we were able to take maternal IQ into account, had detailed information on breastfeeding, clinical assessment of child IQ by a validated tool, as well as a relatively large sample size. Also, we find it relevant that our results are obtained in a population where less than 1% are never breastfed (0.4%), and more than 60% are still breastfed after 6 months, which seems to be among the highest breastfeeding rates in the world in a high-income setting [45]. The observed difference in IQ score in our analyses corresponds well with the results of a recent meta-analysis which reported a pooled effect of 3.5 IQ points associated with breastfeeding, and a slightly smaller difference of 2.2 IQ points in studies that were able to adjust for maternal intelligence [3]. According to Jacobson et al the contribution of maternal intelligence to the child's cognitive ability is both genetic and through a more stimulating rearing environment [46]. Furthermore, studies that fail to include maternal intelligence when attempting to show that the link between breastfeeding and cognitive outcome is not attributable to social factors underestimate a critically important factor [22 46]. Our results thus point to some effect of breastfeeding on child IQ over and above that of parent practices and heredity.

We found no clear dose response relation of breastfeeding duration with child cognitive development in our data; rather, our results point to a difference in IQ of more than 3 points

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between children who are breastfeed for a short period of one month or less compared to those who are breastfed longer. This is in line with the results of a study that used propensity score matching techniques [16] as well as a longitudinal US based study [15]; but stands somewhat in contrast with a meta-analysis from 1999 [8] and a more recent systematic literature review [1] which concluded that cognitive developmental benefits increased with duration of breastfeeding. In our data such a relation was present in the unadjusted analysis only, and we can therefore speculate that effect patterns and sizes may differ between studies depending on other variables that influence IQ, or that previous studies have not investigated such threshold effects of breastfeeding.

In our study sample we categorized the shortest duration as ≤1 month, since very few women reported breastfeeding duration shorter than this, reflecting that by far the majority of mothers in Denmark choose to breastfeed their children. Adding to the difficulty of obtaining an exposure group with shorter duration of breastfeeding is the fact, that women who from the beginning choose not to breastfeed may be different from those who do breastfeed; for example, women who rely on medication for various reasons may choose not to breastfeed because of concerns that medication in the breastmilk may harm the infant. This may explain the recent results from the Dutch Generation R Study (N=3761), which compared non-verbal IQ scores among those 'never breastfed' with different durations of breastfeeding, and found a positive association that was, however, attenuated and no longer statistically significant after adjustment for maternal IQ and several potential confounders [24]. Another explanation may be that they assessed non-verbal intelligence by two sub-tests of a Dutch non-verbal IQ test. We used the WPPSI-R, for which reliability coefficients for the IQs for the present age group are very high (0.90-0.96), although they are likely to be somewhat lower when IQs are based on only three verbal and three performance subtests [35].

Our study sample was originally selected to investigate effects of low to moderate alcohol intake on child development, however, this did not seem to inflict on breastfeeding behaviour, since rates of breastfeeding in our sample were similar to rates in the DNBC overall. The differences between

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women who breastfeed or not can only be addressed entirely in a randomized controlled trial, such as the large PROBIT-study in Belarus, where randomization of clinics and hospitals to breastfeeding promotion resulted in a substantial increase both in breastfeeding exclusivity and duration of any breastfeeding. At age 6.5 years children from the PROBIT-study were followed up (n=13889), and children from the intervention arm scored 7.5 points higher on verbal IQ from the Wechsler Abbreviated Scales of Intelligence, providing strong evidence for a beneficial effect of prolonged breastfeeding on children's cognitive development [5]. Such effects may have a long term impact, as suggested by a Danish observational study, which showed a beneficial association between duration of breastfeeding and adult intelligence in two non-overlapping samples (n=973 men and women, n=2280 men, respectively) [18]. According to Huang et al subsequent schooling and other socialization experiences during adolescence do not eliminate the breastfeeding gap that appears in very early childhood [47]. Furthermore, recent studies have found that the effects of breastfeeding may translate into substantial educational differences in adult life [20] and economic gains to society [48].

Previous studies have suggested three different mechanisms underlying such associations: differences in nutrient compositions between human milk and formula, interplay around the feeding situation, and unidentified factors that correlate with both breastfeeding and neurodevelopment, including residual confounding [18]. With respect to differences in nutrient content, long chained polyunsaturated n-3 fatty acids, and docosahexaenoic acid (DHA) in particular, may be involved. DHA is deemed an essential fatty acid, because it cannot be intrinsically produced and must hence be provided by the diet, with fatty fish as the main source. Results from the Danish National Birth Cohort showed both breastfeeding and maternal fish intake to be independently associated with developmental milestones in the young offspring [49].

It is a main strength of our study that we were able to include the most important confounders: maternal intelligence, parental education and home environment, which in separate analyses have

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been shown to account for 19-29% of the variance in IQ [50 51], also in analyses conducted within the LDPS [52]. Additional strengths to our study include the relatively large number of mother-children pairs in our sample, and the fact that child IQ was assessed by a state of the art clinical intelligence test with high reliability coefficients for IQ for the present age group.

The main limitations of our study are the different possible sources of bias inherent in an observational study design. As is the case in most large cohort studies, participants in the DNBC were generally better off and more health conscious compared to the overall Danish population of pregnant women. However, according to a study by Nøhr et al this selection did not result in biased estimates for selected aetiological associations [53]. Furthermore, at the time the women in the DNBC reported on breastfeeding duration they were not aware of the outcome under study, making it less likely that their reporting was affected by this. Still it may be regarded a weakness, that breastfeeding was reported at two occasions, when children were 6 and 18 months old, requiring mothers to recall their practices used a few months before the actual assessment. Also, we were not able to investigate dose of breastfeeding, since we did not restrict the exposure to exclusive breastfeeding.

The LDPS oversampled mothers with moderate alcohol intake in pregnancy, which we adjusted for by weighing all analyses by the relevant sampling fractions. Furthermore, maternal alcohol use was not strongly associated with poorer performance at 5 years [32 54 55]. We therefore believe it to be unlikely that any of the associations we observe are due to selection bias caused by the sampling in the LDPS. However, it may still be regarded as a limitation, that participants for this sub-study of the DNBC were not selected at random, but by very specific exposure criteria related to maternal behavior during pregnancy. We believe that this has been remedied by our weighing of all analyses by sampling fractions, and that our results can therefore be seen as generalizable to the Danish National Birth Cohort, and the Danish population.

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Associations tended to be attenuated with increasing number of covariates in our analyses, suggesting unadjusted confounding or sparse data bias [56]. As is always the case in observational studies, we cannot exclude that our results are affected by residual confounding or confounding by factors not accounted for in our analyses. Furthermore, there may be postnatal factors influencing child IQ that we have not been able to take into consideration; however, these may not act as true confounders but rather as intermediates, in which case they should not be included in the analyses. Current recommendations from the World Health Organization are that women should breastfeed exclusively for the first six months. Our finding of a 3 point difference in IQ associated with any duration of breastfeeding longer than one month is in support of current recommendations, and is even a relaxed message to mothers who struggle with exclusive breastfeeding. Furthermore, our finding of a somewhat lower, but still significant difference of 1.6 IQ point between those breastfed for 6 months or longer compared to shorter durations underlines the advantage of adhering to the recommendations of the WHO of continued breastfeeding beyond 6 months of age. Although negligible when considered at the individual level, seen from a public health perspective a difference of 3 IQ points must be considered substantial, and smaller effects have previously led to quite conservative precautionary recommendations, for example with respect to adverse effects of maternal exposure to environmental toxicants [57].

In conclusion, in this large sample with high quality assessment of child IQ, we found support of a beneficial association for breastfeeding with child IQ at 5 years of age, while adjusting for maternal IQ and parental education, which only few previous studies have been able to do. Taking the necessary precautions that our results may reflect residual confounding, our findings support current recommendations with respect to breastfeeding in relation to cognitive development of the child.

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Author's contributions

The authors' responsibilities were as follows:

SFO and MS designed research; ELM, UK, SFO, TIH, JO and MS provided essential materials and conducted research; MS and TIH performed statistical analysis; MS, TIH, ELM, UK, SFO interpreted the results. MS wrote paper; MS had primary responsibility for final content. All authors read and approved the final manuscript. All authors had full access to study data. None of the authors had a conflict of interest.

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Figure legend

Figure 1
Overview of the study sample from the Lifestyle During Pregnancy Study

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Table 1: Study participants in the Lifestyle During Pregnancy Study distributed by maternal and child characteristics and by breastfeeding duration (n=1385)

		Breastfeeding duration					
	Overall	**≤1 month**	**2-3 months**	**4-6 months**	**7-9 months**	**≥10 months**	**p[1]**
Maternal age at pregnancy (years)[2]	30.9 (4.4)	30.9 (4.5)	29.9 (4.4)	30.1 (4.2)	30.9 (4.1)	32.0 (4.3)	<0.0001
Parity[2]							0.0003
Nulliparous	676 (48.8)	80 (57.6)	82 (56.2)	122 (49.0)	194 (49.0)	198 (43.5)	
1 child	467 (33.7)	41 (29.5)	50 (34.3)	88 (35.3)	142 (35.9)	146 (32.1)	
≥2 children	242 (17.5)	18 (13.0)	14 (9.6)	39 (15.7)	60 (15.2)	111 (24.4)	
Prepregnancy BMI (kg/m²)[2]	22.6 (4.5)	22.8 (4.1)	23.3 (4.7)	22.7 (4.7)	22.6 (4.0)	22.2 (4.3)	<0.0001
Marital status[2]							0.3
Single	37 (2.7)	4 (2.9)	2 (1.4)	11 (4.4)	7 (1.8)	13 (2.9)	
Cohabiting with partner	1348 (97.3)	135 (97.1)	144 (98.6)	238 (95.6)	389 (98.2)	442 (97.1)	
Alcohol, drinks/wk in pregnancy[2]	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.3
Smoking in pregnancy[2]							<0.0001
Yes	444 (32.1)	54 (38.9)	66 (45.2)	91 (36.6)	102 (25.8)	131 (28.8)	
No	941 (67.9)	85 (61.2)	80 (54.8)	158 (63.5)	294 (74.2)	324 (71.2)	
Postnatal smoking[2]							<0.0001
Yes	449 (32.4)	51 (36.7)	74 (50.7)	97 (39.0)	98 (24.8)	129 (28.4)	
No	936 (67.6)	88 (63.3)	72 (49.3)	152 (61.0)	298 (75.3)	326 (71.7)	
Maternal IQ [2]	100.0 (14.9)	100.4 (14.9)	94.3 (14.8)	97.1 (13.8)	102.2 (14.0)	103.3 (14.9)	<0.0001
Parental education (years)[2]	13.0 (3.0)	13.0 (3.5)	12.3 (3.0)	12.5 (2.5)	13.0 (2.5)	13.5 (2.5)	<0.0001
Child sex[2]							0.5
Girls	654 (47.2)	74 (53.2)	66 (45.2)	115 (46.2)	178 (45.0)	221 (48.6)	
Boys	731 (52.8)	65 (46.8)	80 (54.8)	134 (53.8)	218 (55.1)	234 (51.4)	
Gestational age at birth (weeks)[2]	40.1 (1.5)	40.1 (1.7)	39.8 (1.7)	40.0 (1.5)	40.2 (1.5)	40.1 (1.4)	0.3
Birth weight *z*-score[2]	0.00 (0.97)	0.02 (0.98))	-0.22 (0.93)	0.01 (0.95)	-0.01 (0.90)	0.11 (1.00)	0.005

1 p-value from chi² test (categorical variables: parity, marital status, smoking in pregnancy, postnatal smoking, child sex), F-test one way anova (normally distributed variables: maternal age, maternal IQ, gestational age, *z*-score for birth weight), or Kruskal-Wallis test (non-normally distributed variables: prepregnancy BMI, alcoholic drinks/week in pregnancy, parental education)

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2 Values are mean (SD) (for normally distributed variables: maternal age, maternal IQ, gestational age, *z*-score for birth weight), median (IQR) (for non-normally distributed variables: prepregnancy BMI, alcoholic drinks/week in pregnancy, parental education) or n (%) (for categorical variables: parity, marital status, smoking in pregnancy, postnatal smoking, child sex)

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Table 2: Full scale IQ[1] among 1385 children from the Lifestyle During Pregnancy Study in association with breastfeeding duration

		Crude		Adjusted for core confounders[2]		Adjusted for all potential confounders[2]	
Breastfeeding	Regression α	Regression β	95% CI	Regression β	95% CI	Regression β	95% CI
	101.93						
Per month breastfeeding		0.33	(0.19; 0.47)	0.04	(-0.10; 0.18)	0.07	(-0.08;0.21)
		<0.0001[3]		*0.58*[3]		*0.37*[3]	
<=1 month	98.84	Ref.	-	Ref.	-	Ref.	-
2-3 months		3.92	(1.13;6.71)	3.06	(0.39;5.72)	3.24	(0.61;5.86)
4-6 months		4.37	(1.87;6.87)	2.03	(-0.38;4.44)	1.70	(-0.69;4.08)
7-9 months		6.61	(4.21;9.01)	3.53	(1.18;5.87)	3.36	(1.06;5.66)
≥10 months		7.85	(5.46;10.25)	3.28	(0.88;5.67)	3.41	(1.02;5.80)
		<0.0001[4]		*0.03*[4]		*0.02*[4]	

1. Wechsler Preschool and Primary Scale of Intelligence-Revised.
2. Covariate models; core confounders: Maternal IQ, parental education, smoking during pregnancy, child's age at testing, testing psychologist. All potential confounders: core + maternal age at pregnancy, maternal marital status, maternal parity, sex of child, maternal prepregnant BMI, postnatal parental smoking, health index, home environment, maternal average alcohol intake during pregnancy.
3. *P*-value for the hypothesis of no linear trend in IQ-scores across exposure as continuous.
4. *P*-value for the hypothesis of no difference in IQ-scores across levels of exposure, overall categorical test.

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Table 3: Verbal and Performance scale IQ[1] among 1385 children from the Lifestyle During Pregnancy Study in association with breastfeeding duration

Verbal IQ		Crude		Adjusted for core confounders[2]		Adjusted for all potential confounders[2]	
	Regression αVerbal IQ[1]	Regression β	95% CI	Regression β	95% CI	Regression β	95% CI
	104.09						
Per month breastfeeding		0.20	(0.08; 0.32)	-0.003	(-0.12; 0.12)	0.06	(-0.09;0.22)
		0.001[3]		*0.95*[3]		*0.41*[3]	
<=1 month	100.61	Ref.	-	Ref.	-	Ref.	-
2-3 months		1.77	(-0.61;4.15)	0.88	(-1.41;3.18)	1.04	(-1.24;3.33)
4-6 months		2.79	(0.66;4.92)	1.12	(-0.96;3.20)	1.07	(-1.01;3.15)
7-9 months		4.42	(2.37;6.47)	2.33	(0.30;4.35)	2.19	(0.17;4.20)
≥10 months		4.95	(2.91;6.99)	1.71	(-0.36;3.77)	2.17	(0.08;4.26)
		<0.0001[4]		*0.18*[4]		*0.18*[4]	
Performance IQ		Crude		Adjusted for core confounders[2]		Adjusted for all potential confounders[2]	
	Regression αPerform. IQ[1]	Mean difference	95% CI	Mean difference	95% CI	Mean difference	95% CI
	103.64						
Per month breastfeeding		0.39	(0.22; 0.57)	0.06	(-0.12; 0.24)	0.09	(-0.09;0.27)
		<0.0001[3]		*0.51*[3]		*0.31*[3]	
<=1 month	97.08	Ref.	-	Ref.	-	Ref.	-
2-3 months		5.49	(1.96;9.03)	4.95	(1.55;8.36)	5.30	(1.96;8.63)
4-6 months		5.13	(1.73;8.30)	2.39	(-0.71;5.48)	2.13	(-0.90;5.17)
7-9 months		7.52	(4.48;10.57)	3.84	(0.83;6.84)	3.87	(0.93;6.81)
≥10 months		9.29	(6.26;12.32)	4.06	(0.99;7.13)	4.34	(1.29;7.39)
		<0.0001[4]		*0.03*[4]		*0.009*[4]	

1. Wechsler Preschool and Primary Scale of Intelligence-Revised, verbal and performance scale, respectively.

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2. Covariate models; core confounders: Maternal IQ, parental education, smoking during pregnancy, child's age at testing, testing psychologist. All potential confounders: core + maternal age at pregnancy, maternal marital status, maternal parity, sex of child, maternal prepregnant BMI, postnatal parental smoking, health index, home environment, maternal average alcohol intake during pregnancy.
3. *P*-value for the hypothesis of no linear trend in IQ-scores across exposure as continuous.
4. *P*-value for the hypothesis of no difference in IQ-scores across levels of exposure, overall categorical test.

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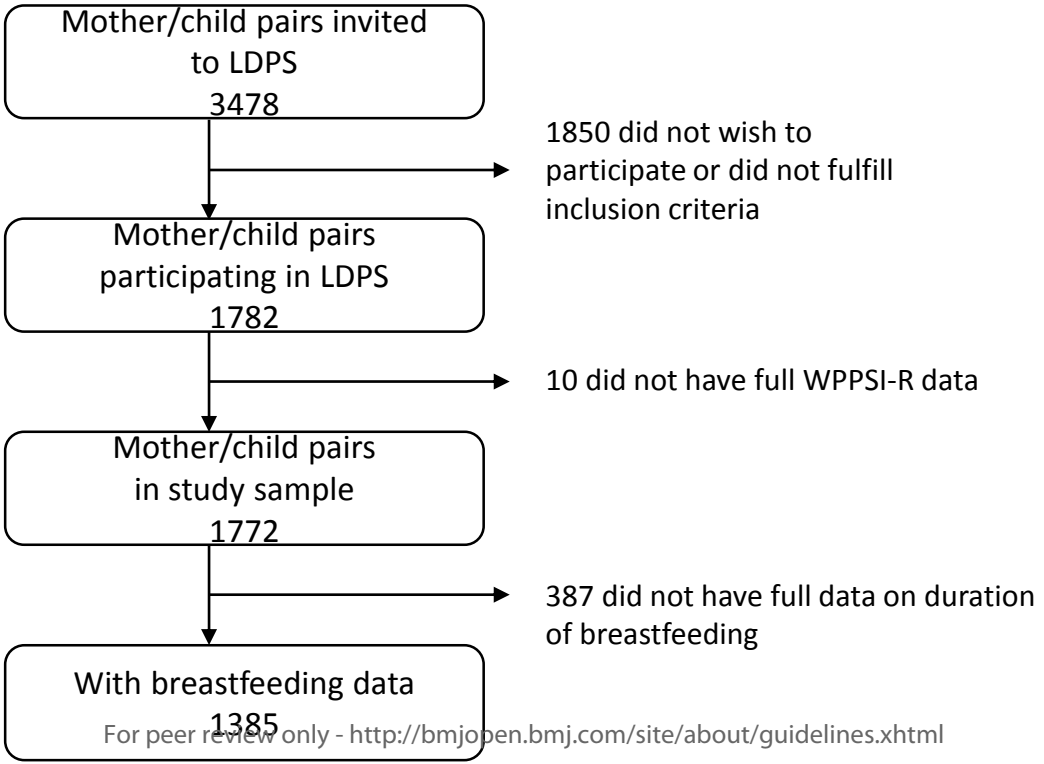

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Supplementary table 1: Full scale IQ[1] among 1385 children from the Lifestyle During Pregnancy Study in association with breastfeeding duration, stratified by child sex

Girls		Crude		Adjusted for core confounders		Adjusted for all potential confounders[2]	
	Regression α	Regression β	95% CI	Regression β	95% CI	Regression β	95% CI
	106.77						
Per month breastfeeding		0.32	(0.11; 0.52)	0.07	(-0.14; 0.27)	-0.04	(-0.26;0.17)
		0.003[3]		*0.52*[3]		*0.70*[3]	
<=1 month	101.89	Ref.	-	Ref.	-	Ref.	-
2-3 months		7.56	(3.34;11.79)	7.10	(3.19;11.01)	6.99	(3.01;10.97)
4-6 months		2.17	(-1.44;5.79)	-0.73	(-4.09;2.63)	-1.12	(-4.58;2.34)
7-9 months		4.30	(0.74;7.86)	-0.25	(-3.64;3.15)	-1.18	(-4.63;2.27)
≥10 months		8.09	(4.59;11.59)	3.25	(-0.10;6.61)	2.01	(-1.45;5.46)
		<0.0001[4]		*<0.0001*[4]		*<0.0001*[4]	
Boys		Crude		Adjusted for core confounders		Adjusted for all potential confounders[2]	
	Regression α	Regression β	95% CI	Regression β	95% CI	Regression β	95% CI
	102.44						
Per month breastfeeding		0.33	(0.15; 0.52)	0.03	(-0.16; 0.22)	0.06	(-0.13;0.25)
		0.0004[3]		*0.75*[3]		*0.52*[3]	
<=1 month	96.53	Ref.	-	Ref.	-	Ref.	-
2-3 months		1.98	(-1.61;5.56)	0.33	(-3.16;3.83)	1.32	(-2.11;4.75)
4-6 months		5.85	(2.52;9.17)	2.87	(-0.41;6.16)	3.28	(0.08;6.48)
7-9 months		8.39	(5.26;11.52)	5.36	(2.27;8.45)	4.98	(1.96;8.00)
≥10 months		7.30	(4.15;10.45)	2.13	(-1.09;5.36)	3.00	(-0.20;6.21)
		<0.0001[4]		*0.0005*[4]		*0.009*[4]	

1. Wechsler Preschool and Primary Scale of Intelligence-Revised.

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2. Covariate models; core confounders: Maternal IQ, parental education, smoking during pregnancy, child's age at testing, testing psychologist. All potential confounders: core + maternal age at pregnancy, maternal marital status, maternal parity, sex of child, maternal prepregnant BMI, postnatal parental smoking, health index, home environment, maternal average alcohol intake during pregnancy.
3. *P*-value for the hypothesis of no linear trend in IQ-scores across exposure as continuous.
4. *P*-value for the hypothesis of no difference in IQ-scores across levels of exposure, overall categorical test.

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STROBE Statement—Checklist of items that should be included in reports of ***cohort 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 X – p. 4
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found X – p. 4-5
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported X – p. 6-7
Objectives	3	State specific objectives, including any prespecified hypotheses X – p. 7
Methods		
Study design	4	Present key elements of study design early in the paper X – p. 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection X – p. 7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up X – p. 7-8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable X – p. 8-10
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 X – p. 7-10
Bias	9	Describe any efforts to address potential sources of bias X – p. 9-12
Study size	10	Explain how the study size was arrived at X – p. 9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why X – p. 8-10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding X – p. 10-12
		(*b*) Describe any methods used to examine subgroups and interactions X – p. 12
		(*c*) Explain how missing data were addressed X – p. 12
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses X – p. 12
Results		

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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 X – p. 9
		(b) Give reasons for non-participation at each stage X – p. 9
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders X – p. 12
		(b) Indicate number of participants with missing data for each variable of interest X – p. 11
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time X – p. 12-13
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 X – p. 13-14, Table 2
		(*b*) Report category boundaries when continuous variables were categorized X – Table 1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses X – p. 13-14
Discussion		
Key results	18	Summarise key results with reference to study objectives X – p. 14
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 X – p. 15,18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence X – p. 15-18
Generalisability	21	Discuss the generalisability (external validity) of the study results X – p. 15-16
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 X – p. 1-2

*Give information separately for exposed and unexposed groups.

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 http://www.strobe-statement.org.

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Is breastfeeding associated with offspring IQ at age 5? Findings from prospective cohort: lifestyle during pregnancy study

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Is breastfeeding associated with offspring IQ at age 5? Findings from prospective cohort: lifestyle during pregnancy study.

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ABSTRACT

Objectives

Breastfeeding is associated with health benefits for both mother and child, but many studies focusing on neurodevelopment have lacked information on important confounders and few randomized trials exist. Our objective was to examine the influence of breastfeeding on child IQ at 5 years of age while taking maternal IQ and other relevant factors into account.

Design

Prospective observational study

Setting

Population based birth cohort in Denmark

Participants

We used data from The Lifestyle During Pregnancy Study 1782 mother-child pairs sampled from the Danish National Birth Cohort (n=101,042).

Outcome measures

Child IQ was assessed at age 5 years by the Wechsler Primary and Preschool Scales of Intelligence-Revised. On the same occasion maternal intelligence was assessed by Wechsler Adult Intelligence Scale and Raven's Standard Progressive Matrices. Exposure data on duration of breastfeeding (n=1385) were extracted from telephone interviews conducted when the child was 6 and 18 months, and analyses were weighted by relevant sampling fractions.

Results

In multivariable linear regression analyses adjusted for potential confounders breastfeeding was associated with child IQ at 5 years (categorical chi^2 test for overall association p=0.03). Compared to children who were breastfed $\leq$1 month, children breastfed for 2-3, 4-6, 7-9 and 10 or more months had 3.06 (95% CI 0.39;5.72), 2.03 (95% CI -0.38;4.44), 3.53 (95% CI 1.18;5.87) and 3.28 (95% CI 0.88;5.67) points higher IQ after adjustment for core confounders, respectively. There was

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no dose-response relation and further analyses indicated that the main difference in IQ was between breastfeeding ≤1 month vs >1 month.

Conclusions

Breastfeeding duration of 1 month or shorter compared to longer periods was associated with approximately 3 points lower IQ, but there was no evidence of a dose-response relation in this prospective birth cohort, where we were able to adjust for some of the most critical confounders, including maternal intelligence.

Article Summary

- Study based on a large population based pregnancy cohort with prospective data assessments
- This study of the association between breastfeeding and child IQ took into account maternal intelligence and home environment, two critical confounders
- Very few women did not breastfeed (6/1385, 0.4%), so it was not possible to investigate this group separately
- Postnatal factors may have influenced child IQ that we have not been able to take into consideration

Funding

The Lifestyle During Pregnancy Study was supported primarily by Centers for Disease Control and Prevention, Atlanta, Georgia, USA, and also from the National Board of Health, the Lundbeck Foundation, Ludvig & Sara Elsass' Foundation, the Augustinus Foundation, and Aase & Ejnar Danielsens Foundation. Furthermore, the study was supported by the European Union (EU) Integrated Research Project EARNEST (FOOD-CT-2005-007036). The Danish National Birth Cohort has been financed by the March of Dimes Birth Defects Foundation, the Danish Heart Association, the Danish Medical Research Council, and the Sygekassernes Helsefond, Danish National Research Foundation, Danish Pharmaceutical Association, Ministry of Health, National

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Board of Health, Statens Serum Institut. The study is part of the research programme of the Center for Fetal Programming (the Danish Council for Strategic Research grant no. 09-067124).
The funding sources had no role in the study design, interpretation of the data, or publication of the results.

Competing Interests

All authors declare no competing interests

Data sharing statement

Data are not available for this study due to the data regulations from the relevant cohort responsible: the Lifestyle During Pregnancy Study and the Danish National Birth Cohort.

Abbreviations

BMI: body mass index

CI: confidence interval

DNBC: Danish National Birth Cohort

FSIQ: Full Scale IQ

IQ: intelligence quotient

IQR: Interquartile range

LDPS: Lifestyle During Pregnancy Study

SD: standard deviation

WPPSI-R: Wechsler Primary and Preschool Scales of Intelligence-Revised

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INTRODUCTION

There is firm evidence for the beneficial effects of breastfeeding on a wide range of maternal and child outcomes [1 2] and to ensure healthy growth and development the World Health Organization recommends that all children are exclusively breastfed for the first six months of life, followed by partial breastfeeding [3].

Since the very early observations by Hoefer and Hardy in 1929 of a positive association for breastfeeding with cognitive performance at age 7-13 years [4], a large body of evidence has provided results in this field. Due to difficulties and ethical concerns intrinsic in designing an intervention targeting breastfeeding duration, few randomized trials exist [5-7]. These have shown beneficial effects of breastfeeding in relation with child neurodevelopmental outcomes, and one of these indicated that exclusive breastfeeding may not confer superior effects compared to breastfeeding and complementary foods introduced at 4 months of age [7]. Several observational studies have shown positive associations for duration of breastfeeding with cognitive developmental benefits in childhood [8-16] and in adult life [17-20]. Differences in nutrient compositions between human milk and formula have been suggested as one of several potential mechanisms underlying a beneficial effect of breastfeeding on cognitive development [18]. In some studies, crude positive associations between breastfeeding and cognitive endpoints were attenuated upon adjustment for potential confounders, including socioeconomic background [21-25], which has made authors conclude that breastfeeding may be a proxy for home environment or parental practices rather than a causal factor in itself [21-27]. However, many studies have been relatively small (n<500), have had limited details on breastfeeding or have not been able to take into account some of the critical confounders including maternal intelligence [9 12 26]. A systematic review and meta-analysis in 2013 concluded that breastfeeding is associated with increased performance in intelligence tests in childhood and adolescence of 3.5 points on average, and that maternal IQ, despite being an important confounder, can account only for part of this association [3]. While the practical implications of such moderate

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differences in intelligence tests can be questioned, a recent study from Brazil [20] indicated that a cognitive advantage by breastfeeding was maintained until adulthood mediating effects on life outcomes including educational attainment and income even in a setting without strong social patterning of breastfeeding [20 28].

The aim of the present study was to assess, in an observational setting, the influence of breastfeeding on child IQ at 5 years of age while taking maternal IQ and home environment into account in the Lifestyle During Pregnancy Study.

SUBJECTS AND METHODS

The aim of the Lifestyle During Pregnancy Study (LDPS) is to examine the relation between maternal lifestyle during pregnancy and offspring neurodevelopment by the age of 5 years [29]. The study is a subsample of the large prospective Danish National Birth Cohort (DNBC) [30]. The DNBC recruited >100,000 pregnant women between 1996 and 2002, and data collection instruments included two pregnancy telephone interviews (in gestational weeks 12 and 30 approximately), two telephone interviews postpartum (at 6 and 18 months postpartum), as well as a detailed food frequency questionnaire (FFQ) which was mailed to the women in gestational week 25 and covered the dietary intake in the previous month [31]. Information on birth outcome was obtained from the Medical Birth Registry.

The LPDS consists of 3478 mother-child dyads selected from the DNBC with oversampling of pregnant women with moderate weekly alcohol intake, alcohol binge drinkers and women with high vs. low fish intake, iron intake and duration of breastfeeding, respectively [29]. A detailed description of the LDPS, including the sampling frame, has been published elsewhere [29 32]. In brief, 3478 mother-child pairs from the DNBC were invited to participate in a follow up around the time when the children turned 5 years. Of the selected dyads 1782 mothers and children (51%) took part in a comprehensive three-hour assessment of neurodevelopment focusing on global cognition, specific

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cognitive functions, and behaviour of the child, and additionally mothers completed tests of adult intelligence (IQ) [29].

Exclusion criteria for the follow-up in LDPS were multiple pregnancies, inability to speak Danish, impaired hearing or vision of the child likely to compromise the ability to perform the cognitive tests, and congenital disorders likely to cause mental retardation, including trisomy 21 [29]. All test procedures were standardised in detail and carried out by 10 trained psychologists. The examinations took place in the four largest cities of Denmark: Copenhagen, Aarhus, Odense and Ålborg [29].

Exposure variables

At both telephone interviews postpartum, when the child was 6 and 18 months, respectively, mothers answered questions about breastfeeding. At the 6 months' interview they reported whether they had never breastfed, were currently breastfeeding or had stopped breastfeeding. Those who were no longer breastfeeding reported the child's age when daily breastfeeding stopped. At 18 months postpartum mothers were asked whether they had breastfed their child beyond 6 months, and if so, whether they were still breastfeeding; if not, they reported the child's age at discontinuation of breastfeeding. Combining the answers from these two data collection instruments we constructed a measure of duration of any breastfeeding, not distinguishing between exclusive and supplemented breastfeeding.

Outcome variables

We used child IQ assessed at the follow up examination at age 5 with the Wechsler Primary and Preschool Scales of Intelligence-Revised (WPPSI-R) [33]. The WPPSI-R is one of the most widely used standardized tests of intelligence for children of 2-7 years of age. In its full form the test battery for WPPSI-R comprises 5 verbal subtests and 5 performance (non-verbal) subtests from

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which verbal IQ, performance IQ, and full scale (FS) IQs are derived. The length of the examination was accommodated to match the age of the study participants (5 years), and therefore a short form of the WPPSI-R was used, which included three verbal (Arithmetic, Information and Vocabulary) and three performance subtests (Block Design, Geometric Design and Object Assembly). Standard procedures were used to prorate IQs from the shortened forms of the tests [34], and since no Danish WPPSI-R norms were available at the time of the study Swedish norms were used to derive scaled scores and IQs [35]. The use of Swedish norms might cause a slight shift in the IQ distribution, and the theoretical mean of 100 (SD 15) cannot necessarily be expected in this sample. However, as the analyses conducted in this study are internal comparisons across exposure, a systematic shift in the IQ distribution should not affect our results.

Mother-child pairs available for analysis

Out of the total number of participants in the LDPS (n=1782), the study sample for these analyses consisted of those 1772 (99.4%) mother-child dyads in the LDPS with full outcome data on the WPPSI-R. Full information on breastfeeding was available for 1385 (78.2%) of these as lined out in **figure 1**.

Covariates

We used information from the prenatal telephone interviews, a questionnaire at the 5-year follow-up examination, and from the Medical Birth Registry and Central Person Registry to define potential confounders that we included in our analyses as covariates. Maternal IQ was assessed at the follow-up examination by two verbal subtests (information and vocabulary) from the Wechsler Adult Intelligence Scale [36] and non-verbal IQ by the Raven's Standard Progressive Matrices [37]. Raw scores of each of the two subtests (information and vocabulary) were standardised based on the results from the full sample. Subsequently scores from each of the two subtests were weighted

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equally into one combined score, and this combined score was then restandardised to a scale with a mean of 100 and SD of 15.

We defined the covariates as follows: parity (0, 1, ≥2 previous births); prenatal maternal smoking (yes, no); maternal pre-pregnancy body mass index (BMI) (weight(kg)/height(m)^2); parental education (average educational length in years for the parents or of mother if that of the father was unavailable); marital status (single during pregnancy or at 5 year follow up, married/cohabitating at both); postnatal parental smoking (yes if mother or father smoked in the home, no if otherwise); maternal average alcohol intake in pregnancy (0, 1-4, 5-8, ≥9 drinks/week); an index of the child's health status (dichotomized as normal or suboptimal in the presence of any illness, diseases, handicaps and/or medication with potential influence on test performance); an index of the quality of postnatal home environment (dichotomized as normal or suboptimal in the presence of two or more of the following seven conditions: not living with a biological parent, changes in caregiver, day care for more than 8 hours/day before age 3, 14+ days of separation from the parents, breakfast irregularity, maternal depression and parental alcohol use above the maximum recommended level by the Danish Health Authority at the time of follow-up of 14 (women) or 21 (men) drinks/week); maternal age at pregnancy (in years); maternal IQ (score), sex of child (girl, boy) and age of child at the follow-up examination. Gestational age was calculated from the last menstrual period provided by the mother at study recruitment (gestation week 6-10) or from the expected date of delivery provided by the woman during the second telephone interview (gestation week 30), which was most often based on ultrasound results [38]. The date of birth was extracted from the Central Person Registry, and the midwife who attended the child's birth recorded birth weight which we extracted from the Medical Birth Registry and calculated sex-specific weight *z*-score at birth on the basis of published reference data [39 40].

The 10 trained psychologists who carried out the testing of the study participants were blinded to the child's exposure status, and we took tester differences into account by the inclusion of a

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categorical variable with 10 levels in the statistical analyses. In a sensitivity analysis tester was taken into consideration in the analysis by a random effect.

Statistical analyses

We used the median (interquartile range (IQR)) to describe skewed variables; mean (standard deviation (SD)) for normally distributed variables; and n/percentage for categorical variables. Throughout, when analyzing associations with continuous measures *p*-values from F tests (type II) are presented, for associations with categorical measures *p*-values from chi^2 test (type III) are presented.

The exposure variable, breastfeeding duration, was examined in relation to child IQ both as a continuous variable (breastfeeding duration in months), and categorized as any breastfeeding ≤1 month, 2-3 months, 4-6 months, 7-9 months or ≥10 months. When analyzing breastfeeding as a continuous measure, we present p-values from F test of trend across months of breastfeeding, when looking at breastfeeding as a categorical variable we present p-values from overall F test of any difference in IQ score across groups of breastfeeding duration.

Furthermore, for supplementary analysis we dichotomized the exposure variable in order to examine the difference in FSIQ according to duration of any breastfeeding ≤1 month vs. >1 month, and ≤6 months vs. >6 months.

We present differences in WPPSI-R FSIQ scores across exposure levels by using multivariable linear regression models to obtain β estimates and corresponding 95% confidence intervals (CIs). In our multivariable analyses we adjusted for potential confounders in two steps: maternal IQ, parental education, maternal smoking during pregnancy, age of child at the follow-up examination and tester were considered core confounders, and we considered this our main analysis.; in a more extensively adjusted model we furthermore included the following potential confounders: maternal age, maternal marital status, parity, sex of child, maternal prepregnancy BMI, postnatal parental

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smoking, health index, home environment and maternal average alcohol intake during pregnancy. Supplementary to this, a non-linear relation between duration of breastfeeding (continuous) and child FSIQ was tested comparing a linear model to a model using restricted cubic splines with 3 knots placed at the 25th, 50th and 75th percentiles [41]. To test for deviations from linearity, we used a likelihood ratio test (P curvature, F test) to compare the linear model with a model fit that based based on restricted cubic splines.

Due to the oversampling of study participants according to certain behavioural exposures, as described above (moderate alcohol use, binge drinking, fish, iron intake and duration of breastfeeding) all analyses were weighted by sampling fractions to account for the complex stratified sampling design [32].

For the 1385 mother-child pairs with data on exposures and outcome, data on child's age at testing and testing psychologist was complete, whereas information on other covariates was missing for approximately 8% of participants (ranging from 7.9% for maternal smoking during pregnancy to 9.5% for maternal prepregnancy BMI). We substituted missing covariate values by multiple imputation using the standard procedure PROC MI in SAS, which utilizes all the information available and imputes 5 different datasets based on which we calculated 5 different sets of effect and variance estimates, and subsequently used the procedure PROC MIANALYZE to compute a combined estimate and standard error for each regression coefficient in our analyses. Results from alternative analytic strategies using complete case and substitution of missing values with mean/mode imputation did not alter our conclusions.

All analyses were carried out using SAS statistical software (version 9.4, SAS Institute, Cary, North Carolina, USA), and all tests were two-sided with statistical significance set to $p<0.05$.

Supplementary analyses

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To avoid overadjustment gestational age and birth weight *z*-score were not included in our main models. As supplementary analysis we furthermore excluded those mother-child pairs who never initiated breastfeeding (n=6). We stratified by child sex, in order to examine potential sex specific effects. We also conducted separate analyses for performance IQ (PIQ) and verbal IQ (VIQ), respectively. Finally, we dichotomised the three IQ outcomes and conducted analyses by logistic regression models presenting chi^2 tests evaluating statistical significance for overall association. Dichotomisation was made using the sample mean minus one SD for the relevant IQ score as described by Eriksen [42](FSIQ dichotomized at 92.5, PIQ at 88.7, VIQ at 94.0) as a cut-off score for subnormal test performance, which implied categorising 209 (15.1%) as subnormal for FSIQ, 232 (16.8%) as subnormal for PIQ, and 229 (16.5%) as subnormal for VIQ.

The study was approved by the DNBC Board of Directors, the DNBC Steering committee, the regional Ethics Committee, the Danish Data Protection Agency, and the CDC Institutional Review Board.

Patient and Public Involvement

Patients were not involved in this study. Results are disseminated to study participants through regular news letters, the website of the DNBC and DNBC on social media.

RESULTS

The children in the LDPS were 60-64 months of age at follow up (mean 5.2 years, range 5.0-5.3 years), and 53% of the children were boys [32]. The mean (SD) FSIQ in the study sample was 105.3 (12.8). The study sample for the present analyses, who had information on breastfeeding (n=1385) did not differ from the full LDPS sample with respect to maternal age (mean (SD) was 30.9 (4.4)

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and 30.9 (4.3) respectively), education (median (IQR) 13.0 (3.0) in both samples), smoking during pregnancy (32.4% and 31.5%, respectively), and cohabitation status in pregnancy (2.7% and 2.9% single, respectively).

Women who breastfed for less than one month compared to 7-9 and more than 10 months were generally younger, they were more likely to be nulliparous (had not previously given birth), have higher BMI, to have been smokers during pregnancy or to have their children be exposed to tobacco smoke postnatally, and have lower IQ (**table 1**). Duration of parental education and birthweight *z*-score was highest for children breastfed for 10 months or longer, and lowest for those who were breastfed for 2-3 months.

Child IQ at the 5-year examination was directly associated with maternal IQ (Spearman r=0.29, p<0.0001), parental education, birthweight *z*-score and gestational age, whereas associations were inverse for parity, prepregnancy BMI, smoking during pregnancy and postnatal smoke exposure (data not shown). Mean IQ was slightly higher for girls compared with boys (supplementary table 1).

Duration of breastfeeding was associated with child IQ at 5 years, even after adjustment for core confounders which included maternal IQ (p from overall categorical test 0.03) (**table 2**). Compared to children who were breastfed one month or less, children who were breastfed for 2-3, 4-6, 7-9 and 10 or more months had 3.06 (95% CI 0.39;5.72), 2.03 (95% CI -0.38;4.44), 3.53 (95% CI 1.18;5.87) and 3.28 (95% CI 0.88;5.67) points higher IQ, respectively. These results remained very similar after more extensive adjustment of potential confounders. When analysing duration of breastfeeding as a continuous variable, the association was statistically significant only in the unadjusted analyses (unadjusted β (95% CI): 0.33 (0.19;0.47), adjusted β: 0.04 (-0.10;0.18)). Furthermore, the estimates for 2-3, 4-6, 7-9, ≥10 months breastfeeding groups were not statistically different from each other (F-test categorical test for overall association omitting mother-child pairs with breastfeeding <= 1 month, p=0.21), indicating that there was no dose-response, but that the

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difference in FSIQ lies between those breastfed ≤ 1 month vs >1 month. Dichotomizing the breastfeeding variable by duration ≤ 1 month vs >1 month resulted in a 2.98 (95% CI 0.86;5.11) point lower FSIQ in adjusted analysis for children who were breastfed ≤1 month vs >1 month, whereas a dichotomization of breastfeeding by duration ≤6 months vs >6 months resulted in a difference of 1.61 (95% CI 0.29;2.93) points. We subsequently tested for a non-linear association using restricted cubic splines, which provided significantly better fit compared to the linear model (p=0.02), and the model with splines was significantly different from the null (p for non-linearity =0.03).

Adjustment for gestational age and z-score for birth weight in supplementary analyses did not alter our study results, and neither did using a random effect for testing psychologist. Exclusion of mother-child pairs that had never initiated breastfeeding (6/1385, 0.4%) did not change our results for the association between breastfeeding and child IQ, but interestingly the mean maternal IQ in the lowest breastfeeding group decreased (from 100.4 to 94.0). When we analysed subscales of IQ, associations were strongest for performance IQ (**table 3**); for verbal IQ effect estimates went in the same direction as for full scale IQ, but were attenuated and not consistently statistically significant across the different adjustment models .

When we stratified for child sex, overall associations were the same and remained statistically significant for both boys and girls, even if confidence intervals widened considerably and effect estimates for the specific breastfeeding groups fluctuated between boys and girls (**supplementary table 1**).

Finally, analyses using dichotomized IQ measures indicated associations in the same direction as the main analyses, but confidence intervals were wide, and associations were generally not statistically significant.

DISCUSSION

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In this relatively large study population which was sampled from the DNBC we investigated associations for duration of breastfeeding with child IQ at age 5 years assessed with the WPPSI-R by a team of trained psychologists. We saw an approximate 3-point difference in IQ between those who were breastfed for one month or less compared to those who were breastfed longer.

Our results are in support of the previous studies that have shown beneficial associations for breastfeeding with cognitive measures in children [9-12 43 44]. It furthermore adds to the current body of literature, since we were able to take maternal IQ into account, had detailed information on breastfeeding, clinical assessment of child IQ by a validated tool, as well as a relatively large sample size. Also, we find it relevant that our results are obtained in a population where less than 1% are never breastfed (0.4%), and more than 60% are still breastfed after 6 months, which seems to be among the highest breastfeeding rates in the world in a high-income setting [45]. The observed difference in IQ score in our analyses corresponds well with the results of a recent meta-analysis which reported a pooled effect of 3.5 IQ points associated with breastfeeding, and a slightly smaller difference of 2.2 IQ points in studies that were able to adjust for maternal intelligence [3]. According to Jacobson et al the contribution of maternal intelligence to the child's cognitive ability is both genetic and through a more stimulating rearing environment [46]. Furthermore, studies that fail to include maternal intelligence when attempting to show that the link between breastfeeding and cognitive outcome is not attributable to social factors underestimate a critically important factor [22 46]. Our results thus point to some effect of breastfeeding on child IQ over and above that of parent practices and heredity.

We found no clear dose response relation of breastfeeding duration with child cognitive development in our data; rather, our results point to a difference in IQ of approximately than 3 points between children who are breastfeed for a short period of one month or less compared to

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those who are breastfed longer. This is in line with the results of a study that used propensity score matching techniques [16] as well as a longitudinal US based study [15]; but stands somewhat in contrast with a meta-analysis from 1999 [8] and a more recent systematic literature review [1] which concluded that cognitive developmental benefits increased with duration of breastfeeding. In our data such a relation was present in the unadjusted analysis only, and we can therefore speculate that effect patterns and sizes may differ between studies depending on other variables that influence IQ, or that previous studies have not investigated such threshold effects of breastfeeding.

In our study sample we categorized the shortest duration as ≤1 month, since very few women reported breastfeeding duration shorter than this, reflecting that by far the majority of mothers in Denmark choose to breastfeed their children. Adding to the difficulty of obtaining an exposure group with shorter duration of breastfeeding is the fact, that women who from the beginning choose not to breastfeed may be different from those who do breastfeed; for example, women who rely on medication for various reasons may choose not to breastfeed because of concerns that medication in the breastmilk may harm the infant. This may explain the recent results from the Dutch Generation R Study (N=3761), which compared non-verbal IQ scores among those 'never breastfed' with different durations of breastfeeding, and found a positive association that was, however, attenuated and no longer statistically significant after adjustment for maternal IQ and several potential confounders [24]. Another explanation may be that they assessed non-verbal intelligence by two sub-tests of a Dutch non-verbal IQ test. We used the WPPSI-R, for which reliability coefficients for the IQs for the present age group are very high (0.90-0.96); in our study they are likely to be somewhat lower since IQs are based on only three verbal and three performance subtests [35].

Our study sample was originally selected to investigate effects of low to moderate alcohol intake on child development, however, this did not seem to inflict on breastfeeding behaviour, since rates of breastfeeding in our sample were similar to rates in the DNBC overall. The differences between women who breastfeed or not can only be addressed entirely in a randomized controlled trial, such

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as the large PROBIT-study in Belarus, where randomization of clinics and hospitals to breastfeeding promotion resulted in a substantial increase both in breastfeeding exclusivity and duration of any breastfeeding. At age 6.5 years children from the PROBIT-study were followed up (n=13889), and children from the intervention arm scored 7.5 points higher on verbal IQ from the Wechsler Abbreviated Scales of Intelligence, providing strong evidence for a beneficial effect of prolonged breastfeeding on children's cognitive development [5]. Such effects may have a long term impact, as suggested by a Danish observational study, which showed a beneficial association between duration of breastfeeding and adult intelligence in two non-overlapping samples (n=973 men and women, n=2280 men, respectively) [18]. According to Huang et al subsequent schooling and other socialization experiences during adolescence do not eliminate the breastfeeding gap that appears in very early childhood [47]. Furthermore, recent studies have found that the effects of breastfeeding may translate into substantial educational differences in adult life [20] and economic gains to society [48].

Previous studies have suggested three different mechanisms underlying such associations: differences in nutrient compositions between human milk and formula, interplay around the feeding situation, and unidentified factors that correlate with both breastfeeding and neurodevelopment, including residual confounding [18]. With respect to differences in nutrient content, long chained polyunsaturated n-3 fatty acids, and docosahexaenoic acid (DHA) in particular, may be involved. DHA is deemed an essential fatty acid, because it cannot be intrinsically produced and must hence be provided by the diet, with fatty fish as the main source. Results from the Danish National Birth Cohort showed both breastfeeding and maternal fish intake to be independently associated with developmental milestones in the young offspring [49].

It is a main strength of our study that we were able to include the most important confounders: maternal intelligence, parental education and home environment, which in separate analyses have been shown to account for 19-29% of the variance in IQ [50 51], also in analyses conducted within the

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LDPS [52]. Additional strengths to our study include the relatively large number of mother-children pairs in our sample, and the fact that child IQ was assessed by a state of the art clinical intelligence test with high reliability coefficients for IQ for the present age group.

The main limitations of our study are the different possible sources of bias inherent in an observational study design. As is the case in most large cohort studies, participants in the DNBC were generally better off and more health conscious compared to the overall Danish population of pregnant women. However, according to a study by Nøhr et al this selection did not result in biased estimates for selected aetiological associations [53]. Furthermore, at the time the women in the DNBC reported on breastfeeding duration they were not aware of the outcome under study, making it less likely that their reporting was affected by this. Still, it may be regarded a weakness, that breastfeeding was reported at two occasions, when children were 6 and 18 months old, requiring mothers to recall their practices used a few months before the actual assessment. Also, we were not able to investigate dose of breastfeeding, since we did not restrict the exposure to exclusive breastfeeding.

The LDPS oversampled mothers with moderate alcohol intake in pregnancy, which we adjusted for by weighing all analyses by the relevant sampling fractions. Furthermore, maternal alcohol use was not strongly associated with poorer performance at 5 years [32 54 55]. We therefore believe it to be unlikely that any of the associations we observe are due to selection bias caused by the sampling in the LDPS. However, it may still be regarded as a limitation, that participants for this sub-study of the DNBC were not selected at random, but by very specific exposure criteria related to maternal behavior during pregnancy. We believe that this has been remedied by our weighing of all analyses by sampling fractions, and that our results can therefore be seen as generalizable to the Danish National Birth Cohort, and the Danish population.

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Associations tended to be attenuated with increasing number of covariates in our analyses, suggesting unadjusted confounding or sparse data bias [56]. As is always the case in observational studies, we cannot exclude that our results are affected by residual confounding or confounding by factors not accounted for in our analyses. Furthermore, there may be postnatal factors influencing child IQ that we have not been able to take into consideration; however, these may not act as true confounders but rather as intermediates, in which case they should not be included in the analyses. Current recommendations from the World Health Organization are that women should breastfeed exclusively for the first six months. Our finding of a 3 point difference in IQ associated with any duration of breastfeeding longer than one month is in support of current recommendations, and is even a relaxed message to mothers who struggle with exclusive breastfeeding. Furthermore, our finding of a somewhat lower, but still significant difference of 1.6 IQ point between those breastfed for 6 months or longer compared to shorter durations underlines the advantage of adhering to the recommendations of the WHO of continued breastfeeding beyond 6 months of age. Although negligible when considered at the individual level, seen from a public health perspective a difference of 3 IQ points must be considered substantial, and smaller effects have previously led to quite conservative precautionary recommendations, for example with respect to adverse effects of maternal exposure to environmental toxicants [57].

Conclusion

In conclusion, in this large sample with high quality assessment of child IQ, we found support of a beneficial association for breastfeeding with child IQ at 5 years of age, while adjusting for maternal IQ and parental education, which only few previous studies have been able to do. Taking the necessary precautions that our results may reflect residual confounding, our findings support current recommendations with respect to breastfeeding in relation to cognitive development of the child.

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Author's contributions

The authors' responsibilities were as follows:

SFO and MS designed research; ELM, UK, SFO, TIH, JO and MS provided essential materials and conducted research; MS and TIH performed statistical analysis; MS, TIH, ELM, UK, SFO interpreted the results. MS wrote paper; MS had primary responsibility for final content. All authors read and approved the final manuscript. All authors had full access to study data. None of the authors had a conflict of interest.

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Figure legend

Figure 1
Overview of the study sample from the Lifestyle During Pregnancy Study

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Table 1: Study participants in the Lifestyle During Pregnancy Study distributed by maternal and child characteristics and by breastfeeding duration (n=1385)

		Breastfeeding duration					
	Overall	**≤1 month**	**2-3 months**	**4-6 months**	**7-9 months**	**≥10 months**	**p[1]**
Maternal age at pregnancy (years)[2]	30.9 (4.4)	30.9 (4.5)	29.9 (4.4)	30.1 (4.2)	30.9 (4.1)	32.0 (4.3)	<0.0001
Parity[2]							0.0003
Nulliparous	676 (48.8)	80 (57.6)	82 (56.2)	122 (49.0)	194 (49.0)	198 (43.5)	
1 child	467 (33.7)	41 (29.5)	50 (34.3)	88 (35.3)	142 (35.9)	146 (32.1)	
≥2 children	242 (17.5)	18 (13.0)	14 (9.6)	39 (15.7)	60 (15.2)	111 (24.4)	
Prepregnancy BMI (kg/m²)[2]	22.6 (4.5)	22.8 (4.1)	23.3 (4.7)	22.7 (4.7)	22.6 (4.0)	22.2 (4.3)	<0.0001
Marital status[2]							0.3
Single	37 (2.7)	4 (2.9)	2 (1.4)	11 (4.4)	7 (1.8)	13 (2.9)	
Cohabiting with partner	1348 (97.3)	135 (97.1)	144 (98.6)	238 (95.6)	389 (98.2)	442 (97.1)	
Alcohol, drinks/wk in pregnancy[2]	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.3
Smoking in pregnancy[2]							<0.0001
Yes	444 (32.1)	54 (38.9)	66 (45.2)	91 (36.6)	102 (25.8)	131 (28.8)	
No	941 (67.9)	85 (61.2)	80 (54.8)	158 (63.5)	294 (74.2)	324 (71.2)	
Postnatal smoking[2]							<0.0001
Yes	449 (32.4)	51 (36.7)	74 (50.7)	97 (39.0)	98 (24.8)	129 (28.4)	
No	936 (67.6)	88 (63.3)	72 (49.3)	152 (61.0)	298 (75.3)	326 (71.7)	
Maternal IQ [2]	100.0 (14.9)	100.4 (14.9)	94.3 (14.8)	97.1 (13.8)	102.2 (14.0)	103.3 (14.9)	<0.0001
Parental education (years)[2]	13.0 (3.0)	13.0 (3.5)	12.3 (3.0)	12.5 (2.5)	13.0 (2.5)	13.5 (2.5)	<0.0001
Child sex[2]							0.5
Girls	654 (47.2)	74 (53.2)	66 (45.2)	115 (46.2)	178 (45.0)	221 (48.6)	
Boys	731 (52.8)	65 (46.8)	80 (54.8)	134 (53.8)	218 (55.1)	234 (51.4)	
Gestational age at birth (weeks)[2]	40.1 (1.5)	40.1 (1.7)	39.8 (1.7)	40.0 (1.5)	40.2 (1.5)	40.1 (1.4)	0.3
Birth weight *z*-score[2]	0.00 (0.97)	0.02 (0.98))	-0.22 (0.93)	0.01 (0.95)	-0.01 (0.90)	0.11 (1.00)	0.005

1 p-value from chi² test (categorical variables: parity, marital status, smoking in pregnancy, postnatal smoking, child sex), F-test one way anova (normally distributed variables: maternal age, maternal IQ, gestational age, *z*-score for birth weight), or Kruskal-Wallis test (non-normally distributed variables: prepregnancy BMI, alcoholic drinks/week in pregnancy, parental education)

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2 Values are mean (SD) (for normally distributed variables: maternal age, maternal IQ, gestational age, *z*-score for birth weight), median (IQR) (for non-normally distributed variables: prepregnancy BMI, alcoholic drinks/week in pregnancy, parental education) or n (%) (for categorical variables: parity, marital status, smoking in pregnancy, postnatal smoking, child sex)

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Table 2: Full scale IQ[1] among 1385 children from the Lifestyle During Pregnancy Study in association with breastfeeding duration

		Crude		Adjusted for core confounders[2]		Adjusted for all potential confounders[2]	
Breastfeeding	Regression α	Regression β	95% CI	Regression β	95% CI	Regression β	95% CI
	101.93						
Per month breastfeeding		0.33	(0.19; 0.47)	0.04	(-0.10; 0.18)	0.07	(-0.08;0.21)
		<0.0001[3]		*0.58*[3]		*0.37*[3]	
<=1 month	98.84	Ref.	-	Ref.	-	Ref.	-
2-3 months		3.92	(1.13;6.71)	3.06	(0.39;5.72)	3.24	(0.61;5.86)
4-6 months		4.37	(1.87;6.87)	2.03	(-0.38;4.44)	1.70	(-0.69;4.08)
7-9 months		6.61	(4.21;9.01)	3.53	(1.18;5.87)	3.36	(1.06;5.66)
≥10 months		7.85	(5.46;10.25)	3.28	(0.88;5.67)	3.41	(1.02;5.80)
		<0.0001[4]		*0.03*[4]		*0.02*[4]	

1. Wechsler Preschool and Primary Scale of Intelligence-Revised.
2. Covariate models; core confounders: Maternal IQ, parental education, smoking during pregnancy, child's age at testing, testing psychologist. All potential confounders: core + maternal age at pregnancy, maternal marital status, maternal parity, sex of child, maternal prepregnant BMI, postnatal parental smoking, health index, home environment, maternal average alcohol intake during pregnancy.
3. *P*-value for the hypothesis of no linear trend in IQ-scores across exposure as continuous.
4. *P*-value for the hypothesis of no difference in IQ-scores across levels of exposure, overall categorical test.

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Table 3: Verbal and Performance scale IQ[1] among 1385 children from the Lifestyle During Pregnancy Study in association with breastfeeding duration

Verbal IQ		Crude		Adjusted for core confounders[2]		Adjusted for all potential confounders[2]	
	Regression αVerbal IQ[1]	Regression β	95% CI	Regression β	95% CI	Regression β	95% CI
	104.09						
Per month breastfeeding		0.20	(0.08; 0.32)	-0.003	(-0.12; 0.12)	0.06	(-0.09;0.22)
		0.001[3]		*0.95*[3]		*0.41*[3]	
<=1 month	100.61	Ref.	-	Ref.	-	Ref.	-
2-3 months		1.77	(-0.61;4.15)	0.88	(-1.41;3.18)	1.04	(-1.24;3.33)
4-6 months		2.79	(0.66;4.92)	1.12	(-0.96;3.20)	1.07	(-1.01;3.15)
7-9 months		4.42	(2.37;6.47)	2.33	(0.30;4.35)	2.19	(0.17;4.20)
≥10 months		4.95	(2.91;6.99)	1.71	(-0.36;3.77)	2.17	(0.08;4.26)
		<0.0001[4]		*0.18*[4]		*0.18*[4]	
Performance IQ		Crude		Adjusted for core confounders[2]		Adjusted for all potential confounders[2]	
	Regression αPerform. IQ[1]	Mean difference	95% CI	Mean difference	95% CI	Mean difference	95% CI
	103.64						
Per month breastfeeding		0.39	(0.22; 0.57)	0.06	(-0.12; 0.24)	0.09	(-0.09;0.27)
		<0.0001[3]		*0.51*[3]		*0.31*[3]	
<=1 month	97.08	Ref.	-	Ref.	-	Ref.	-
2-3 months		5.49	(1.96;9.03)	4.95	(1.55;8.36)	5.30	(1.96;8.63)
4-6 months		5.13	(1.73;8.30)	2.39	(-0.71;5.48)	2.13	(-0.90;5.17)
7-9 months		7.52	(4.48;10.57)	3.84	(0.83;6.84)	3.87	(0.93;6.81)
≥10 months		9.29	(6.26;12.32)	4.06	(0.99;7.13)	4.34	(1.29;7.39)
		<0.0001[4]		*0.03*[4]		*0.009*[4]	

1. Wechsler Preschool and Primary Scale of Intelligence-Revised, verbal and performance scale, respectively.

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2. Covariate models; core confounders: Maternal IQ, parental education, smoking during pregnancy, child's age at testing, testing psychologist. All potential confounders: core + maternal age at pregnancy, maternal marital status, maternal parity, sex of child, maternal prepregnant BMI, postnatal parental smoking, health index, home environment, maternal average alcohol intake during pregnancy.
3. *P*-value for the hypothesis of no linear trend in IQ-scores across exposure as continuous.
4. *P*-value for the hypothesis of no difference in IQ-scores across levels of exposure, overall categorical test.

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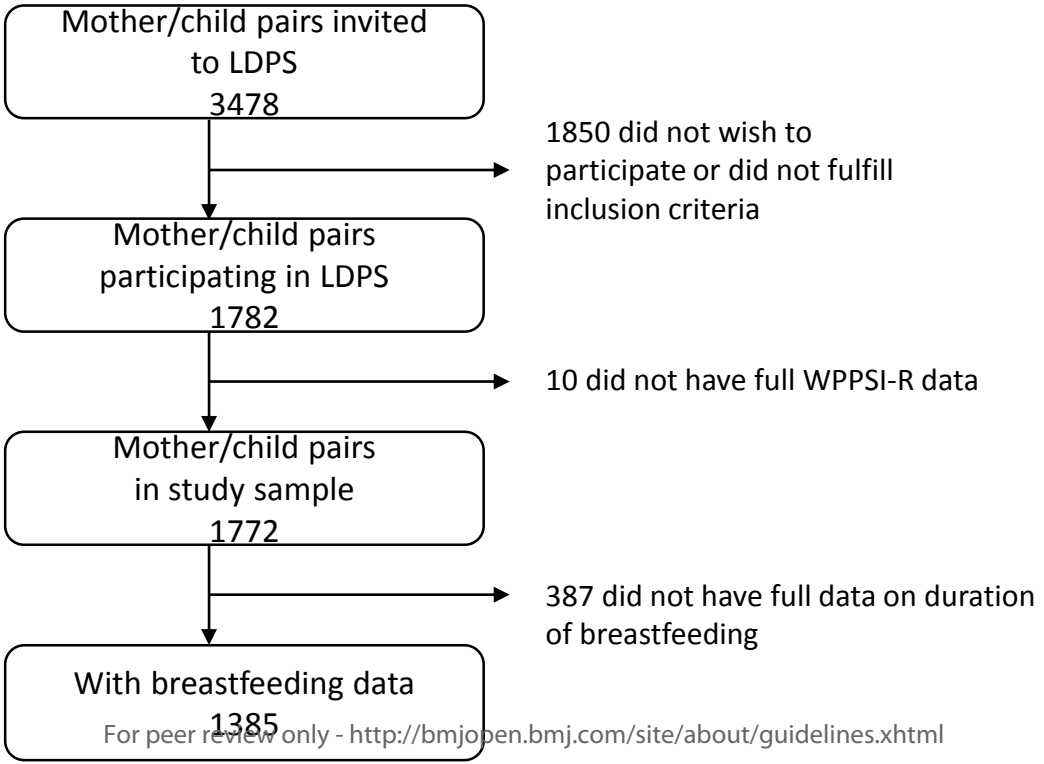

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Supplementary table 1: Full scale IQ[1] among 1385 children from the Lifestyle During Pregnancy Study in association with breastfeeding duration, stratified by child sex

Girls		Crude		Adjusted for core confounders		Adjusted for all potential confounders[2]	
	Regression α	Regression β	95% CI	Regression β	95% CI	Regression β	95% CI
	106.77						
Per month breastfeeding		0.32	(0.11; 0.52)	0.07	(-0.14; 0.27)	-0.04	(-0.26;0.17)
		0.003[3]		*0.52*[3]		*0.70*[3]	
<=1 month	101.89	Ref.	-	Ref.	-	Ref.	-
2-3 months		7.56	(3.34;11.79)	7.10	(3.19;11.01)	6.99	(3.01;10.97)
4-6 months		2.17	(-1.44;5.79)	-0.73	(-4.09;2.63)	-1.12	(-4.58;2.34)
7-9 months		4.30	(0.74;7.86)	-0.25	(-3.64;3.15)	-1.18	(-4.63;2.27)
≥10 months		8.09	(4.59;11.59)	3.25	(-0.10;6.61)	2.01	(-1.45;5.46)
		<0.0001[4]		*<0.0001*[4]		*<0.0001*[4]	
Boys		Crude		Adjusted for core confounders		Adjusted for all potential confounders[2]	
	Regression α	Regression β	95% CI	Regression β	95% CI	Regression β	95% CI
	102.44						
Per month breastfeeding		0.33	(0.15; 0.52)	0.03	(-0.16; 0.22)	0.06	(-0.13;0.25)
		0.0004[3]		*0.75*[3]		*0.52*[3]	
<=1 month	96.53	Ref.	-	Ref.	-	Ref.	-
2-3 months		1.98	(-1.61;5.56)	0.33	(-3.16;3.83)	1.32	(-2.11;4.75)
4-6 months		5.85	(2.52;9.17)	2.87	(-0.41;6.16)	3.28	(0.08;6.48)
7-9 months		8.39	(5.26;11.52)	5.36	(2.27;8.45)	4.98	(1.96;8.00)
≥10 months		7.30	(4.15;10.45)	2.13	(-1.09;5.36)	3.00	(-0.20;6.21)
		<0.0001[4]		*0.0005*[4]		*0.009*[4]	

1. Wechsler Preschool and Primary Scale of Intelligence-Revised.

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2. Covariate models; core confounders: Maternal IQ, parental education, smoking during pregnancy, child's age at testing, testing psychologist. All potential confounders: core + maternal age at pregnancy, maternal marital status, maternal parity, sex of child, maternal prepregnant BMI, postnatal parental smoking, health index, home environment, maternal average alcohol intake during pregnancy.
3. *P*-value for the hypothesis of no linear trend in IQ-scores across exposure as continuous.
4. *P*-value for the hypothesis of no difference in IQ-scores across levels of exposure, overall categorical test.

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STROBE Statement—Checklist of items that should be included in reports of ***cohort 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 X – p. 4
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found X – p. 4-5
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported X – p. 6-7
Objectives	3	State specific objectives, including any prespecified hypotheses X – p. 7
Methods		
Study design	4	Present key elements of study design early in the paper X – p. 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection X – p. 7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up X – p. 7-8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable X – p. 8-10
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 X – p. 7-10
Bias	9	Describe any efforts to address potential sources of bias X – p. 9-12
Study size	10	Explain how the study size was arrived at X – p. 9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why X – p. 8-10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding X – p. 10-12
		(*b*) Describe any methods used to examine subgroups and interactions X – p. 12
		(*c*) Explain how missing data were addressed X – p. 12
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses X – p. 12
Results		

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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 X – p. 9
		(b) Give reasons for non-participation at each stage X – p. 9
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders X – p. 12
		(b) Indicate number of participants with missing data for each variable of interest X – p. 11
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time X – p. 12-13
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 X – p. 13-14, Table 2
		(*b*) Report category boundaries when continuous variables were categorized X – Table 1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses X – p. 13-14
Discussion		
Key results	18	Summarise key results with reference to study objectives X – p. 14
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 X – p. 15,18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence X – p. 15-18
Generalisability	21	Discuss the generalisability (external validity) of the study results X – p. 15-16
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 X – p. 1-2

*Give information separately for exposed and unexposed groups.

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 http://www.strobe-statement.org.

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