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The apparent breastfeeding paradox in very preterm infants: relationship between breastfeeding, early weight gain, and neurodevelopment based on results from two cohorts, EPIPAGE and LIFT.

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ABSTRACT

CONTEXT: Supplementation of breast milk is difficult once infants suckle the breast and is often discontinued at end of hospitalization and after discharge. Thus breastfed preterm infants are exposed to an increased risk of nutritional deficit with a possible consequence on neurodevelopmental outcome.

OBJECTIVE: To assess the relationship between breastfeeding at time of discharge, weight gain during hospitalization and neurodevelopmental outcome.

DESIGN: Observational cohort study.

SETTING: Two large independent population based cohorts of very preterm infants: the LIFT and the EPiPAGE cohorts.

PATIENTS: 2925 very preterm infants alive at discharge.

MAIN OUTCOME MEASURE: Suboptimal neurodevelopmental outcome, defined as a score in the lower tercile, using Age and Stages Questionnaire at 2 years in LIFT and K-ABC test at 5 years in EPiPAGE. Two propensity scores for breastfeeding at discharge, one for each cohort, were used to reduce bias.

RESULTS. Breastfeeding at time of discharge concerned only 278/1733 (16%) in LIFT and 409/2163 (19%) infants in EPiPAGE cohort. Breastfeeding is significantly associated with an increased risk of losing one weight Z-Score during hospitalization (LIFT: n=1463, aOR=2.51 [1.87-3.36]; EPiPAGE: n=1417, aOR=1.55 [1.14-2.12]) and with a decreased risk for a suboptimal neurodevelopmental assessment (LIFT: n=1463, aOR=0.63 [0.45-0.87]; EPiPAGE: n=1441, aOR=0.65 [0.47-0.89] and an increased chance of having a head circumference Z-score higher than 0.5 at two years in LIFT cohort. (n=1276, aOR=1.43, [1.02-2.02]) and at five years in EPiPAGE cohort, (n=1412, aOR=1.47, [1.10-1.95]).

CONCLUSION. The observed better neurodevelopment in spite of suboptimal initial weight gain could be termed the ‘apparent breastfeeding paradox’ in very preterm infants. Regardless of the mechanisms involved the current data provide encouragement for the use of breastfeeding in preterm infants.
Summary

ARTICLE FOCUS: Supplementation of breast milk is difficult once infants suckle the breast and is often discontinued at end of hospitalization and after discharge. Thus breastfed preterm infants are exposed to an increased risk of nutritional deficit with a possible consequence on neurodevelopmental outcome.

KEY MESSAGES: Breastfeeding at time of discharge is associated with a better neurodevelopment in spite of suboptimal initial weight gain. Regardless of the mechanisms involved the current data provide encouragement for the use of breastfeeding in preterm infants

STRENGTHS OF THIS STUDY: The study includes large population data from two different cohorts at different times. Large proportion of population included in Follow up study.

LIMITATIONS OF THE STUDY: It is an observational study hence associated deficiencies. Moreover, we did not address the mechanisms.
INTRODUCTION

Breastfeeding is universally recommended for the feeding of term infants (1). Regarding very preterm infants, less than 32 weeks of gestation, exclusive breastfeeding is a debated topic since supplementation is required to ensure optimal growth during initial hospitalization but is difficult once the preterm infant can suckle the breast (2).

Suboptimal nutrition with insufficient growth during hospitalization in neonatal intensive care unit is associated with later cognitive dysfunction (3,4). In extremely preterm infants, growth velocity during hospitalization exerts a significant, and possibly independent, effect on neurodevelopment and growth outcomes at 18 to 22 months' corrected age (3). In preterm infants, follow up studies showed that at 7 years of age, preterm infants fed standard formula demonstrated neurocognitive impairment with a significant reduction in IQ, compared with infants fed with enriched formula(4).

Breastfeeding with supplementation during initial hospitalization improves cognitive outcome at 30 months of corrected age in extremely preterm infants (5). Human milk indeed requires nutrient fortification to meet the protein and mineral needs of the rapidly growing preterm infant (6). During hospitalization, as the baby receives mother-milk through a gastric tube it is easy to use a milk fortifier to maintain adequate growth. Once the preterm infant can suckle the breast, however, the use of a milk fortifier is not easy, as it disrupts the routine of breastfeeding. As a consequence, mother milk supplementation is often discontinued at the end of hospitalization and hospital discharge, and this discontinuation exposes the infants to an increased risk of nutritional deficit (7). Thus, breastfeeding at time of discharge could be associated with less weight gain during neonatal hospitalization and during the weeks following discharge. This is why exclusive breastfeeding remains a matter of debate in preterm infants.

The aim of the current study was to assess the complex relationship between breastfeeding at time of discharge, weight gain during neonatal hospitalization and neurodevelopment at 2 or 5 years using data from two independent large cohorts of very preterm infants of less than 33 weeks' gestation covering the late 1990s and mid-2000s. The secondary objective was assessment of growth (weight, height, head circumference) at 2 and 5 years.
Methods

Data Source and Patients:

Date source is constituted of two cohorts: EPIPAGE (1) and LIFT (Loire Infant Follow-up Team) (8) cohorts with recruitment over two distinct periods. EPIPAGE is a prospective population-based cohort study including all infants born between 22 and 32 weeks of gestation in 1997 in the maternity wards of nine French regions accounting for about one third of all births in France. Among infants born in 1997, who survived and eligible for the follow up (n=2282), we included all infants whose status regarding breastfeeding at time of discharge was known (n=2163). LIFT cohort is a cohort of infants born in one region (Pays de la Loire, a region in Western France) and enrolled in the regional follow-up network. Among surviving very preterm infants with a gestational age less than 33 weeks of gestation, born between January 1, 2003 and June 30, 2008 and enrolled in LIFT cohort (n=1857), we included all children whose status regarding breastfeeding at time of discharge was known (n=1733) (Fig 1). Each cohort was registered to the French CNIL. For EPIPAGE cohort, parents were told about the study and given written information in the maternity or neonatal unit, and verbal consent was provided to the medical team in charge of the study at recruitment. For LIFT cohort, a written consent was obtained at enrollment.

Developmental assessment

In the EPIPAGE cohort, the neuropsychological assessment was performed using the Kaufman Assessment Battery for Children (K-ABC) at five years of age (9). Neuropsychological assessment was performed by trained psychologists when appropriate for the patient's condition and when accepted by the patient. The K-ABC yields four global test scores. The Mental Processing Composite scale, which is considered to be equivalent to intelligence quotient (IQ), is a global measure of cognitive ability. This scale is standardized to a mean of 100 (SD 15). An MPC in the lower tercile (score of less than 85) was considered as an index of suboptimal neurodevelopment.

In the LIFT cohort, neuro-developmental assessment was performed using Age and stages questionnaires (ASQ) (10), a questionnaire completed by parents at a corrected age of two years. This questionnaire includes five parts, among which three are related to cognitive development: communication, problem solving, and personal social interactions. The sum of the 5 partial scores was calculated, and subject population was split in 3 terciles of global ASQ...
score. Being in the lower tercile (ASQ score less than 220) was considered an index of suboptimal neurodevelopment.

**Growth assessment**

To assess growth, we used measurements performed at birth, discharge, and 6 months, two years and 5 years of age in EPIPAGE cohort, birth, discharge, 9 months and 2 years in LIFT cohort. We calculated Z-Score by using LMS method. We used reference growth curves for which LMS parameters have been published for weight, height and head circumference. For birth and discharge (up to 41 weeks of postmenstrual age) measurements, we used Olsen’s preterm infant growth chart (11). For the few preterm infants discharged after 41 weeks of postmenstrual age, and for follow-up period measurements, we used World Health Organization (WHO) growth curves (12). Weight gain during hospitalization was assessed as the difference of weight Z-score between discharge and birth.

**Statistical Analysis**

Means and standard deviations are reported for continuous variables and frequencies for categorical variables. Anova and χ² or Fisher test if necessary, were used to compare infant characteristics and 5-year outcome between the groups of infants who were breast fed or formula fed at time of discharge.

The propensity score method was used to reduce bias in assessing the relation between breastfeeding at discharge and cognitive outcomes (13-14). The propensity score is defined as a conditional probability, between 0 and 1, that a subject will be “breastfed at discharge” based on an observed group of covariates. This score is then used just as if it were the only confounding covariate. Thus, the collection of predictors is collapsed into a single predictor, which may better adjust covariates between the groups and reduce bias. Two full non-parsimonious logistic regression models were developed to derive a propensity score for breast feeding at discharge, one for each cohort. These models included true confounders: variables that are potentially associated with both mode of feeding and with outcome (15,16). These variables included for the Epipage cohort: characteristics of the mothers (age, maternal body mass index (17), level of maternal education, socioeconomic status, number of children at home), characteristics of the newborns (gestational age, Z-Score of birth weight, presence of a congenital malformation), characteristics of pregnancy (antenatal corticosteroids, multiple pregnancy, complication of pregnancy), place of birth (inborn/outborn, region of birth),
characteristics of neonatal hospitalization (cranial ultrasound abnormalities, patent ductus arteriosus, necrotizing enterocolitis, neonatal surgery, bronchopulmonary dysplasia defined by supplemental oxygen requirement at 36 weeks, duration of mechanical ventilation, length of hospital stay). Patients with missing data were excluded from multivariable analysis. For the LIFT cohort, the variables included in propensity score calculation were similar but less numerous, including characteristics of the mother, characteristics of the newborn and neonatal hospitalization. The Hosmer-Lemeshow goodness-of-fit test and the area under the curve were used to assess each model fit.

First, we studied crude associations between breastfeeding at discharge and suboptimal neurodevelopment assessment and then the same associations after adjustment for the propensity score, gestational age and birth weight Z-score. Due to the known relationship between gestational age, weight gain and suboptimal development, these variables were included as confounding factors in the multivariable model even if they are already included in the calculation of propensity score, as suggested by other authors (14). We used logistic regression models for univariate and multivariate analyses (16). We estimated the crude and adjusted odds ratio, and its 95% confidence interval, of a suboptimal neurodevelopment score, i.e. to be in the lower tercile of MPC (less than 85) at 5 years in EPIPAGE cohort or ASQ (less than 220) at 2 years corrected age in LIFT cohort, associated with breastfeeding at discharge.

Secondly, we studied crude associations between breastfeeding and postnatal weight gain during neonatal hospitalization and thereafter, up to 2 years in LIFT cohort and 5 years in EPIPAGE cohort. Finally we studied the relationship between weight gain during neonatal hospitalization and suboptimal neurodevelopment assessment. Moreover, we assessed growth (weight, height, head circumference) at 6 months, 2 and 5 years in EPIPAGE and at 9 months, and two years in LIFT cohort. All p values were based on two-sided tests. All analyses were performed using SPSS 15.0 (SPSS INC, Chicago, IL).

Results

Patients Characteristics.

The populations enrolled in EPIPAGE (n =2163) and LIFT cohorts (n=1733) were very similar with a small albeit significant difference in gestational age (29.9 ± 2.0 vs. 29.8 ± 2.1 weeks, p=0.01), birth weight (1380 ± 395 vs. 1340 ± 396 g, p=0.01) postnatal weight gain (0.98 ± 0.79 vs. 0.83 ± 0.82 loss of weight Z-score, p=0.001) but not in length of hospital stay (60.2 ± 31.7 vs. 58.7 ± 32.2 days, p=0.13). The proportion of breastfed infants was a little bit higher in
EPIPAGE cohort (19%) than in LIFT cohort (16%), p=0.02. In both cohorts breastfeeding was associated with some characteristics of the mothers, pregnancy, newborns and neonatal hospitalization (Table 1). Among the 2163 children of EPIPAGE cohort, 1753 (81%) were followed up to 5 years and 1462 (68%) assessed by trained psychologists. Among the 1733 of LIFT cohort, 1587 (85%) were followed up to two years of corrected age and we obtained ASQ for 1463 children (79%).

**Propensity Score** was calculated in each cohort. In Epipage cohort, it was possible to calculate the propensity score for 2130 of the 2163 very preterm infants who were alive at hospital discharge and whose status regarding breast feeding status at time of discharge was known. The propensity scores ranged from 0.0005 to 0.722. The Hosmer-Lemeshow test was 7.2, p=0.51. The ROC curve area was 0.72 ± 0.01. In LIFT cohort, the propensity score was calculated for all preterm infants. For each variable, if necessary, a subgroup with unknown data was constituted. The propensity scores ranged from 0.007 to 0.747. The Hosmer-Lemeshow test was 6.2, p=0.63. The ROC curve area was 0.71 ± 0.01. Variables significantly associated with breastfeeding at discharge were very similar in both cohorts (table 2).

**Exposure to Breastfeeding at Time of Discharge and neurodevelopmental Outcome**

In both cohorts, breastfeeding was associated with a significant reduction of risk for a suboptimal neurodevelopmental assessment at 2 years of corrected age (LIFT cohort) and at 5 years, (Table 3). Moreover, The K-ABC Mental Processing Composite score increased as a function of the corrected age at which infants were weaned off breastfeeding (Fig 2). In each cohort, breastfeeding was consistently associated with a reduction in the risk for suboptimal neurodevelopmental assessment before and after adjustment for gestational age, birth weight Z-score and sex, and propensity score (Table 3).

**Weight gain during neonatal hospitalization and exposure to breastfeeding.**

Breastfeeding was associated with an increased risk of losing one weight Z-Score during hospitalization, before adjustment in LIFT cohort, and after adjustment in both cohorts (Table 4).The loss in weight Z-Score during NICU hospitalization was similar in both cohorts in breastfed infants but not in non breastfed infants: the loss was less pronounced in the most recent cohort (Table 1).

**Weight gain during neonatal hospitalization, exposure to breastfeeding, and neuro...**
developmental outcome.

Restricted intrauterine growth (i.e., lower birth weight Z Score) was associated before and after adjustment for gestational age and sex with a suboptimal neurodevelopmental assessment in both cohorts. Postnatal weight gain during neonatal hospitalization was significantly associated with a suboptimal neurodevelopmental assessment only in LIFT cohort but not in EPIPAGE cohort (Table 5), with and without adjustment for gestational age, birth weight Z-score, sex, breastfeeding and propensity score.

Growth after neonatal hospitalization.
In contrast with data on initial growth, from 2 years of corrected age, weight, height and head circumference were significantly higher in preterm infants who had been breastfed at the time of discharge in both LIFT and Epipage cohorts (Fig 2). After adjustment for weight Z Score at birth, sex and propensity score, breastfeeding at discharge was significantly associated to an increased chance of having a head circumference Z-score higher than 0.5 at five years in EPIPAGE cohort, (n=1412, aOR=1.47, [1.10-1.95]) and at two years of corrected age in LIFT cohort, (n=1276, aOR=1.43, [1.02-2.02]).

DISCUSSION
In two independent cohorts of preterm infants, breastfeeding at discharge was associated with a reduction in the risk for a suboptimal neurodevelopmental assessment at two (LIFT cohort) or five years of age (EPIPAGE cohort) despite a higher risk for suboptimal weight gain (loss of one weight Z-Score) during neonatal hospitalization in these breastfed infants. The observed improved neurodevelopment in spite of suboptimal initial weight gain could be termed the ‘apparent breastfeeding paradox’ in very preterm infants.

Such an observation is indeed a paradox as several earlier studies have documented an association between suboptimal early postnatal nutrition with insufficient weight gain during hospital stay and later cognitive dysfunction. In extremely preterm infants, faster weight gain in NICU was associated with improved outcome in terms of neurodevelopment and growth at 18 to 22 months’ corrected age. (3) Nevertheless, this paradox is probably only an apparent paradox because weight gain during hospitalization is a poor predictor of the quality of growth, as it does not provide any insight into the changes in body composition. In infants with a very low birth weight, enhanced postnatal growth is also associated with a better later neurodevelopmental outcome (18), especially regarding postnatal growth in head circumference, an index of brain
growth (19,20). Yet the “neuroprotective” effects of improved growth may only be mild and mainly concern growth over the first few weeks of hospital stay (21). In the two cohorts used in the current analysis, the role of postnatal weight gain was slightly different. In the EPIPAGE cohort, we did not observe any association between postnatal weight gain during hospitalization and sub-optimal neurodevelopmental score at five years of age (Table 5). In the LIFT cohort, after adjustment for propensity score, a significant association was found between initial weight gain rate and neurodevelopment. In contrast, in both cohorts a negative association was obvious between birthweight Z-score and suboptimal neuro-development (Table 5). Such difference between the 2 cohorts could be in relation with the significantly better initial neonatal weight gain rate in the LIFT cohort, compared with the EPIPAGE cohort, as a matter of fact, the loss in weight Z-score was less in the most recent cohort, presumably due to improvement in care routines between the two periods (1997 vs. 2003) in the NICUs in France (22). Thanks to the improvement in nutritional management in the second period, the role of postnatal nutrition, adjusted for breastfeeding, may have become detectable in the more recent LIFT cohort.

Beneficial effects of breastfeeding on cognitive skills and behavioral scores have been demonstrated previously in term (23,24), preterm (25), and extremely preterm infants (5). Multiple biases may, however, interfere, particularly maternal socioeconomic and educational status: in the two cohorts, upper social status was obviously associated with a higher chance for being breastfed at discharge, and for an optimal neurodevelopmental score. In term infants, after control for biases (26)—particularly using the method of sibling comparison which automatically controls for any confounding factors that are the same for each of the siblings in a pair (27)—the role of breastfeeding has been found to be either not significant or modest. In preterm infants, observational studies have shown that preterm infants whose mothers chose to breastfeed reached a higher intelligence quotient at 8 years of age. Interestingly, infants whose mothers were willing to breastfeed but failed to do so had the same IQ as those whose mothers elected not to breastfeed (25). In preterm infants, observational studies found feeding with human milk to be associated with a better outcome (5). Eight randomized studies were included in a Cochrane review (28). Only 2 old randomized studies using unfortified donor human milk reported a comparison of developmental outcome between unfortified human milk and formula feeding. Follow-up of the infants who participated in these two trials did not find a significant effect on long-term growth parameters or neurodevelopmental outcomes. Most of other parameters reported by the randomized studies comparing formula to human milk were in favor of breastmilk, except for weight gain during hospitalization. Moreover, among children who had been enrolled in one of such studies Lucas et al observed 15 years later, that breastmilk consumption was associated with a lower blood pressure in children born prematurely,
suggesting a long-term beneficial effects of breastmilk in preterm infants (29). Taken together, data from the literature, and from the current study are consistent with a benefit of breastmilk consumption on developmental outcome.

At the time of discharge, very preterm infants have accumulated deficits in energy, protein and minerals, and, due to their early discharge, still have higher nutrient requirements than healthy appropriate-for-gestational age term infants. Despite the vast body of published recommendations on the nutrition of preterm infants, there is little data on optimal nutritional management after discharge of breastfed preterm infants (30). No randomized studies can be ethically performed to determine whether feeding preterm infants following hospital discharge with nutrient-enriched formula milk versus human breast milk affects growth and development. The conclusion of a Cochrane review (2) is that “mothers who wish to breast feed, and their health care advisors, would require very clear evidence that feeding with a nutrient-enriched formula milk had major advantages for their infants before electing not to feed (or to reduce feeding) with maternal breast milk, and that evidence from trials that compared feeding preterm infants following hospital discharge with nutrient-enriched versus standard formula milk demonstrating an effect on growth or development”. The need to contribute an answer to the nagging, disturbing question, ‘Does exclusive breastfeeding at the time of discharge influence outcome?’ was the very incentive to perform the current study. Our analysis of two large cohorts of preterm infant with relatively long term follow-up, used the propensity score method as a means to control as much as possible for potential confounders. From such analysis we obtained clear-cut evidence that despite an increased risk for suboptimal early weight gain (increased risk for the loss of one weight Z-Score) during neonatal hospitalization, breastfeeding at discharge is associated with a better outcome after adjustment for potential confounders by using propensity score.

The main weakness of our study is the observational design. Despite the use of propensity score we cannot ensure all potential confounders were eliminated. For instance, the very ability of an infant to suckle may be associated with a less sick infant, and the variables used in adjustment may not be entirely independent either of each other or of the outcome. Moreover, how much fortifier was given could not, unfortunately, be retrieved from either of the two databases, and thus some important variable such as caloric intake during neonatal intensive care unit stay could not be included in the analysis. Similarly, we did not address the mechanisms. For instance, the effect of breastfeeding for term infants has been attributed to bias (31), including the complex relationship between weight gain and timing of weaning. Such bias is unlikely to be relevant for our population since the preterm infants with the slower weight
gain rate were those who were not weaned at discharge. The slower weight gain during neonatal intensive care unit stay therefore likely reflects a biological effect rather than bias. Several potential mechanisms can be proposed to explain the better outcome in breastfed infants: mother-child interaction (better bonding, better care given by parents), the effect of specific nutrients contained in breastmilk such as polyunsaturated fatty acids, prebiotic oligosaccharides, etc. Regardless of mechanisms we did observe the same fact in two distinct cohorts, with the same magnitude. By adjustment for propensity score, sex, and birth weight Z-score, we were indeed able to observe the apparent paradox of a better neurodevelopmental outcome, despite a lower early weight gain. These adjustments are necessary because a complex relationship exists between breastfeeding, birth weight, gestational age, birth weight Z-score, and postnatal weight gain during hospitalization.

In conclusion, the neurodevelopment of premature infants is likely to benefit from feeding unsupplemented mother’s milk after discharge, and these data from two cohorts are indeed reassuring, because in spite of a lesser weight gain during hospitalization, we observed a better neurodevelopmental outcome in the breastfed groups. The present report suggests breastfeeding should be recommended at the time of discharge. As the rate of exclusive breastfeeding at time of discharge is low—less than 30% in Europe (32)—strategies to facilitate breastfeeding at discharge must be developed (33). Moreover, when adjusted on breastfeeding, postnatal weight gain has a positive effect on neurodevelopmental outcome as observed in LIFT cohort; so the question about the putative benefit of human milk supplementation after discharge remains open. This suggests that more research is warranted about human milk composition and the potential benefit of human milk supplementation at time of discharge is warranted in the future.

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Author Contributions: Conceived and designed the experiments: JCR, DD, MK PYA. Analyzed the data: JCR DD MK PYA CYB. Wrote the paper: JCR DD PYA. Data acquisition: JCR, CF, JCP, CS, DM,
BB,US. Revision and critical review of the manuscript: JCR, DD, JCP, AL, OC, DM, US.

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**Data Sharing:** Our data are public, and available for a research project after evaluation of this project. LIFT data can be requested near JC Rozé. EPIPAGE data can be requested near PY Ancel.

**Competing Interests:** None.
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Figure 1. Flow charts.
Figure 2. The K-ABC Mental Processing Composite score (mean, SD) at five years as a function of breastfeeding status at time of discharge and corrected age (*) at which infants were weaned off breastfeeding, in EPIPAGE cohort.
‡ p adjusted for propensity score.
Figure 3. Weight, head circumference and height expressed as Z-score at discharge, 6 or 9 months and 2 years of corrected age (EPIPAGE and LIFT cohorts), and at 5 years (EPIPAGE cohort) according to breastfeeding at discharge. Weight measurements was known for 1460, 1430, 973, 873, 1447 infants at birth, discharge, 6 months, 2 and 5 years respectively in EPIPAGE, and for 1463, 1463, 1341 and 1297 infants at birth, discharge, 9 months and 2 years respectively in LIFT cohort.
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DESIGN: Observational cohort study.

SETTING: Two large independent population based cohorts of very preterm infants: the LIFT and the EPIPAGE cohorts

PATIENTS: 2925 very preterm infants alive at discharge

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INTRODUCTION

Breastfeeding is universally recommended for the feeding of term infants (1). Regarding very preterm infants, less than 32 weeks of gestation, exclusive breastfeeding is a debated topic since supplementation is required to ensure optimal growth during initial hospitalization but is difficult once the preterm infant can suckle the breast (2).

Suboptimal nutrition with insufficient growth during hospitalization in neonatal intensive care unit is associated with later cognitive dysfunction (3,4). In extremely preterm infants, growth velocity during hospitalization exerts a significant, and possibly independent, effect on neurodevelopment and growth outcomes at 18 to 22 months’ corrected age (3). In preterm infants, follow up studies showed that at 7 years of age, preterm infants fed standard formula demonstrated neurocognitive impairment with a significant reduction in IQ, compared with infants fed with enriched formula(4).

Breastfeeding with supplementation during initial hospitalization improves cognitive outcome at 30 months of corrected age in extremely preterm infants (5). Human milk indeed requires nutrient fortification to meet the protein and mineral needs of the rapidly growing preterm infant (6). During hospitalization, as the baby receives mother-milk through a gastric tube it is easy to use a milk fortifier to maintain adequate growth. Once the preterm infant can suckle the breast, however, the use of a milk fortifier is not easy, as it disrupts the routine of breastfeeding. As a consequence, mother milk supplementation is often discontinued at the end of hospitalization and hospital discharge, and this discontinuation exposes the infants to an increased risk of nutritional deficit (7). Thus, breastfeeding at time of discharge could be associated with less weight gain during neonatal hospitalization and during the weeks following discharge. This is why exclusive breastfeeding remains a matter of debate in preterm infants.

The aim of the current study was to assess the complex relationship between breastfeeding at time of discharge, weight gain during neonatal hospitalization and neurodevelopment at 2 or 5 years using data from two independent large cohorts of very preterm infants of less than 33 weeks’ gestation covering the late 1990s and mid-2000s. The secondary objective was assessment of growth (weight, height, head circumference) at 2 and 5 years.
Methods

Data Source and Patients:

Date source is constituted of two cohorts: EPIPAGE (1) and LIFT (Loire Infant Follow-up Team) (8) cohorts with recruitment over two distinct periods. EPIPAGE is a prospective population-based cohort study including all infants born between 22 and 32 weeks of gestation in 1997 in the maternity wards of nine French regions accounting for about one third of all births in France. Among infants born in 1997, who survived and eligible for the follow up (n=2282), we included all infants whose status regarding breastfeeding at time of discharge was known (n=2163). LIFT cohort is a cohort of infants born in one region (Pays de la Loire, a region in Western France) and enrolled in the regional follow-up network. Among surviving very preterm infants with a gestational age less than 33 weeks of gestation, born between January 1, 2003 and June 30, 2008 and enrolled in LIFT cohort (n=1857), we included all children whose status regarding breastfeeding at time of discharge was known (n=1733) (Fig 1). Each cohort was registered to the French CNIL. For EPIPAGE cohort, parents were told about the study and given written information in the maternity or neonatal unit, and verbal consent was provided to the medical team in charge of the study at recruitment. For LIFT cohort, a written consent was obtained at enrollment.

Developmental assessment

In the EPIPAGE cohort, the neuropsychological assessment was performed using the Kaufman Assessment Battery for Children (K-ABC) at five years of age (9). Neuropsychological assessment was performed by trained psychologists when appropriate for the patient’s condition and when accepted by the patient. The K-ABC yields four global test scores. The Mental Processing Composite scale, which is considered to be equivalent to intelligence quotient (IQ), is a global measure of cognitive ability. This scale is standardized to a mean of 100 (SD 15). An MPC in the lower tercile (score of less than 85) was considered as an index of suboptimal neurodevelopment.

In the LIFT cohort, neuro-developmental assessment was performed using Age and stages questionnaires (ASQ) (10), a questionnaire completed by parents at a corrected age of two years. This questionnaire includes five parts, among which three are related to cognitive development: communication, problem solving, and personal social interactions. The sum of the 5 partial scores was calculated, and subject population was split in 3 terciles of global ASQ.
score. Being in the lower tercile (ASQ score less than 220) was considered an index of suboptimal neurodevelopment.

### Growth assessment

To assess growth, we used measurements performed at birth, discharge, and 6 months, two years and 5 years of age in EPIPAGE cohort, birth, discharge, 9 months and 2 years in LIFT cohort. We calculated Z-Score by using LMS method. We used reference growth curves for which LMS parameters have been published for weight, height and head circumference. For birth and discharge (up to 41 weeks of postmenstrual age) measurements, we used Olsen’s preterm infant growth chart (11). For the few preterm infants discharged after 41 weeks of postmenstrual age, and for follow-up period measurements, we used World Health Organization (WHO) growth curves (12). **Weight gain** during hospitalization was assessed as the difference of weight Z-score between discharge and birth.

### Statistical Analysis

Means and standard deviations are reported for continuous variables and frequencies for categorical variables. Anova and $\chi^2$ or Fisher test if necessary, were used to compare infant characteristics and 5-year outcome between the groups of infants who were breast fed or formula fed at time of discharge.

The propensity score method was used to reduce bias in assessing the relation between breastfeeding at discharge and cognitive outcomes (13-14). The propensity score is defined as a conditional probability, between 0 and 1, that a subject will be “breastfed at discharge” based on an observed group of covariates. This score is then used just as if it were the only confounding covariate. Thus, the collection of predictors is collapsed into a single predictor, which may better adjust covariates between the groups and reduce bias. Two full non-parsimonious logistic regression models were developed to derive a propensity score for breast feeding at discharge, one for each cohort. These models included true confounders: variables that are potentially associated with both mode of feeding and with outcome (15,16). These variables included for the Epipage cohort: characteristics of the mothers (age, maternal body mass index (17), level of maternal education, socioeconomic status, number of children at home), characteristics of the newborns (gestational age, Z-Score of birth weight, presence of a congenital malformation), characteristics of pregnancy (antenatal corticosteroids, multiple pregnancy, complication of pregnancy), place of birth (inborn/outborn, region of birth),
characteristics of neonatal hospitalization (cranial ultrasound abnormalities, patent ductus arteriosus, necrotizing enterocolitis, bronchopulmonary dysplasia defined by supplemental oxygen requirement at 36 weeks, duration of mechanical ventilation, length of hospital stay). Patients with missing data were excluded from multivariable analysis. For the LIFT cohort, the variables included in propensity score calculation were similar but less numerous, including characteristics of the mother, characteristics of the newborn and neonatal hospitalization. The Hosmer-Lemeshow goodness-of-fit test and the area under the curve were used to assess each model fit.

First, we studied crude associations between breastfeeding at discharge and suboptimal neurodevelopment assessment and then the same associations after adjustment for the propensity score, gestational age and birth weight Z-score. Due to the known relationship between gestational age, weight gain and suboptimal development, these variables were included as confounding factors in the multivariable model even if they are already included in the calculation of propensity score as suggested by other authors. We used logistic regression models for univariate and multivariate analyses. We estimated the crude and adjusted odds ratio, and its 95% confidence interval, of a suboptimal neurodevelopment score, i.e. to be in the lower tercile of MPC (less than 85) at 5 years in EPIPAGE cohort or ASQ (less than 220) at 2 years corrected age in LIFT cohort. Finally, we studied the relationship between weight gain during neonatal hospitalization and thereafter, up to 2 years in the EPIPAGE cohort and 5 years in the LIFT cohort, associated with breastfeeding at discharge. Moreover, we assessed growth and development in the EPIPAGE and LIFT cohorts. All p values were based on two-sided tests. All analyses were performed using SPSS 15.0 (SPSS INC, Chicago, IL).

Results

Patients Characteristics.

The populations enrolled in EPIPAGE (n = 2163) and LIFT cohorts (n = 1733) were very similar with a small albeit significant difference in gestational age (29.9 ± 2.0 vs. 29.8 ± 2.1 weeks, p = 0.01), birth weight (3300 ± 395 vs. 3369 ± 396 g, p = 0.01) and postnatal weight gain (0.98 ± 0.79 vs. 0.83 ± 0.82 kg, p = 0.19). The proportion of breastfed infants was a little bit higher in the EPIPAGE cohort (60.2 ± 31.7 vs. 56.7 ± 32.2 days, p = 0.19).
EPIPAGE cohort (19%) than in LIFT cohort (16%), p=0.02. In both cohorts breastfeeding was associated with some characteristics of the mothers, pregnancy, newborns and neonatal hospitalization (Table 1). Among the 2163 children of EPIPAGE cohort, 1753 (81%) were followed up to 5 years and 1462 (68%) assessed by trained psychologists. Among the 1733 of LIFT cohort, 1587 (85%) were followed up to two years of corrected age and we obtained ASQ for 1463 children (79%).

Propensity Score was calculated in each cohort. In Epipage cohort, it was possible to calculate the propensity score for 2130 of the 2163 very preterm infants who were alive at hospital discharge and whose status regarding breast feeding status at time of discharge was known. The propensity scores ranged from 0.0005 to 0.722. The Hosmer-Lemeshow test was 7.2, p=0.51. The ROC curve area was 0.72 ± 0.01. In LIFT cohort, the propensity score was calculated for all preterm infants. For each variable, if necessary, a subgroup with unknown data was constituted. The propensity scores ranged from 0.007 to 0.747. The Hosmer-Lemeshow test was 6.2, p=0.63. The ROC curve area was 0.71 ± 0.01. Variables significantly associated with breastfeeding at discharge were very similar in both cohorts (table 2).

Exposure to Breastfeeding at Time of Discharge and neurodevelopmental Outcome

In both cohorts, breastfeeding was associated with a significant reduction of risk for a suboptimal neurodevelopmental assessment at 2 years of corrected age (LIFT cohort) and at 5 years, (Table 3). Moreover, The K-ABC Mental Processing Composite score increased as a function of the corrected age at which infants were weaned off breastfeeding (Fig 2). In each cohort, breastfeeding was consistently associated with a reduction in the risk for suboptimal neurodevelopmental assessment before and after adjustment for gestational age, birth weight Z-score and sex, and propensity score (Table 3).

Weight gain during neonatal hospitalization and exposure to breastfeeding.

Breastfeeding was associated with an increased risk of losing one weight Z-Score during hospitalization, before adjustment in LIFT cohort, and after adjustment in both cohorts (Table 4). The loss in weight Z-Score during NICU hospitalization was similar in both cohorts in breastfed infants but not in non breastfed infants: the loss was less pronounced in the most recent cohort (Table 1).

Weight gain during neonatal hospitalization, exposure to breastfeeding, and neuro
Developmental outcome.

Restricted intrauterine growth (i.e., lower birth weight Z Score) was associated before and after adjustment for gestational age and sex with a suboptimal neurodevelopmental assessment in both cohorts. Postnatal weight gain during neonatal hospitalization was significantly associated with a suboptimal neurodevelopmental assessment only in LIFT cohort but not in in EPIPAGE cohort (Table5), with and without adjustment for gestational age, birth weight Z-score, sex, breastfeeding and propensity score).

Growth after neonatal hospitalization.

In contrast with data on initial growth, from 2 years of corrected age, weight, height and head circumference were significantly higher in preterm infants who had been breastfed at the time of discharge in both LIFT and Epipage cohorts (Fig 2). After adjustment for weight Z Score at birth, sex and propensity score, breastfeeding at discharge was significantly associated to an increased chance of having a head circumference Z-score higher than 0.5 at five years in EPIPAGE cohort, (n=1412, aOR=1.47,[1.10-1.95]) and at two years of corrected age in LIFT cohort, (n=1276, aOR=1.43, [1.02-2.02]).

DISCUSSION

In two independent cohorts of preterm infants, breastfeeding at discharge was associated with a reduction in the risk for a suboptimal neurodevelopmental assessment at two (LIFT cohort) or five years of age (EPIPAGE cohort) despite a higher risk for suboptimal weight gain (loss of one weight Z-Score) during neonatal hospitalization in these breastfed infants. The observed improved neurodevelopment in spite of suboptimal initial weight gain could be termed the ‘apparent breastfeeding paradox’ in very preterm infants.

Such an observation is indeed a paradox as several earlier studies have documented an association between suboptimal early postnatal nutrition with insufficient weight gain during hospital stay and later cognitive dysfunction. In extremely preterm infants, faster weight gain in NICU was associated with improved outcome in terms of neurodevelopment and growth at 18 to 22 months’ corrected age. (3) Nevertheless, this paradox is probably only an apparent paradox because weight gain during hospitalization is a poor predictor of the quality of growth, as it does not provide any insight into the changes in body composition. In infants with a very low birth weight, enhanced postnatal growth is also associated with a better later neurodevelopmental outcome (18), especially regarding postnatal growth in head circumference, an index of brain
growth (19,20). Yet the “neuroprotective” effects of improved growth may only be mild and mainly concern growth over the first few weeks of hospital stay (21). In the two cohorts used in the current analysis, the role of postnatal weight gain was slightly different. In the EPIPAGE cohort, we did not observe any association between postnatal weight gain during hospitalization and sub-optimal neurodevelopmental score at five years of age (Table 5). In the LIFT cohort, after adjustment for propensity score, a significant association was found between initial weight gain rate and neurodevelopment. In contrast, in both cohorts a negative association was obvious between birthweight Z-score and suboptimal neuro-development (Table 5). Such difference between the 2 cohorts could be in relation with the significantly better initial neonatal weight gain rate in the LIFT cohort, compared with the EPIPAGE cohort, as a matter of fact, the loss in weight Z-score was less in the most recent cohort, presumably due to improvement in care routines between the two periods (1997 vs. 2003) in the NICUs in France (22). Thanks to the improvement in nutritional management in the second period, the role of postnatal nutrition, adjusted for breastfeeding, may have become detectable in the more recent LIFT cohort.

Beneficial effects of breastfeeding on cognitive skills and behavioral scores have been demonstrated previously in term (23,24), preterm (25), and extremely preterm infants (5). Multiple biases may, however, interfere, particularly maternal socioeconomic and educational status: in the two cohorts, upper social status was obviously associated with a higher chance for being breastfed at discharge, and for an optimal neurodevelopmental score. In term infants, after control for biases (26)–particularly using the method of sibling comparison which automatically controls for any confounding factors that are the same for each of the siblings in a pair (27)—the role of breastfeeding has been found to be either not significant or modest. In preterm infants, observational studies have shown that preterm infants whose mothers chose to breastfeed reached a higher intelligence quotient at 8 years of age. Interestingly, infants whose mothers were willing to breastfeed but failed to do so had the same IQ as those whose mothers elected not to breastfeed (25). In preterm infants, observational studies found feeding with human milk to be associated with a better outcome (5). Eight randomized studies were included in a Cochrane review (28). Only 2 old randomized studies using unfortified donor human milk reported a comparison of developmental outcome between unfortified human milk and formula feeding. Follow-up of the infants who participated in these two trials did not find a significant effect on long-term growth parameters or neurodevelopmental outcomes. Most of other parameters reported by the randomized studies comparing formula to human milk were in favor of breastmilk, except for weight gain during hospitalization. Moreover, among children who had been enrolled in one of such studies Lucas et al observed 15 years later, that breastmilk consumption was associated with a lower blood pressure in children born prematurely,
suggesting a long-term beneficial effects of breastmilk in preterm infants (29). Taken together, data from the literature, and from the current study are consistent with a benefit of breastmilk consumption on developmental outcome.

At the time of discharge, very preterm infants have accumulated deficits in energy, protein and minerals, and, due to their early discharge, still have higher nutrient requirements than healthy appropriate-for-gestational age term infants. Despite the vast body of published recommendations on the nutrition of preterm infants, there is little data on optimal nutritional management after discharge of breastfed preterm infants (30). No randomized studies can be ethically performed to determine whether feeding preterm infants following hospital discharge with nutrient-enriched formula milk versus human breast milk affects growth and development. The conclusion of a Cochrane review (2) is that “mothers who wish to breast feed, and their health care advisors, would require very clear evidence that feeding with a nutrient-enriched formula milk had major advantages for their infants before electing not to feed (or to reduce feeding) with maternal breast milk, and that evidence from trials that compared feeding preterm infants following hospital discharge with nutrient-enriched versus standard formula milk demonstrating an effect on growth or development “. The need to contribute an answer to the nagging, disturbing question, ‘Does exclusive breastfeeding at the time of discharge influence outcome?’ was the very incentive to perform the current study. Our analysis of two large cohorts of preterm infant with relatively long term follow-up, used the propensity score method as a means to control as much as possible for potential confounders. From such analysis we obtained clear-cut evidence that despite an increased risk for suboptimal early weight gain (increased risk for the loss of one weight Z-Score) during neonatal hospitalization, breastfeeding at discharge is associated with a better outcome after adjustment for potential confounders by using propensity score.

The main weakness of our study is the observational design. Despite the use of propensity score we cannot ensure all potential confounders were eliminated. For instance, the very ability of an infant to suckle may be associated with a less sick infant, and the variables used in adjustment may not be entirely independent either of each other or of the outcome. Moreover, how much fortifier was given could not, unfortunately, be retrieved from either of the two databases, and thus some important variable such as caloric intake during neonatal intensive care unit stay could not be included in the analysis. Similarly, we did not address the mechanisms. For instance, the effect of breastfeeding for term infants has been attributed to bias (31), including the complex relationship between weight gain and timing of weaning. Such bias is unlikely to be relevant for our population since the preterm infants with the slower weight...
gain rate were those who were not weaned at discharge. The slower weight gain during neonatal intensive care unit stay therefore likely reflects a biological effect rather than bias. Several potential mechanisms can be proposed to explain the better outcome in breastfed infants: mother-child interaction (better bonding, better care given by parents), the effect of specific nutrients contained in breastmilk such as polyunsaturated fatty acids, prebiotic oligosaccharides, etc. Regardless of mechanisms we did observe the same fact in two distinct cohorts, with the same magnitude. By adjustment for propensity score, sex, and birth weight Z-score, we were indeed able to observe the apparent paradox of a better neurodevelopmental outcome, despite a lower early weight gain. These adjustments are necessary because a complex relationship exists between breastfeeding, birth weight, gestational age, birth weight Z-score, and postnatal weight gain during hospitalization.

In conclusion, the neurodevelopment of premature infants is likely to benefit from feeding unsupplemented mother’s milk after discharge, and these data from two cohorts are indeed reassuring, because in spite of a lesser weight gain during hospitalization, we observed a better neurodevelopmental outcome in the breastfed groups. The present report suggests breastfeeding should be recommended at the time of discharge. As the rate of exclusive breastfeeding at time of discharge is low—less than 30% in Europe (32)—strategies to facilitate breastfeeding at discharge must be developed (33). Moreover, when adjusted on breastfeeding, postnatal weight gain has a positive effect on neurodevelopmental outcome as observed in LIFT cohort; so the question about the putative benefit of human milk supplementation after discharge remains open. This suggests that more research is warranted about human milk composition and the potential benefit of human milk supplementation at time of discharge is warranted in the future.

**Funding**: Epipage cohort was supported by grants from INSERM (French National Institute of Health and Medical Research), the Directorate General for Health of the Ministry for Social Affairs. Lift cohort was supported by grants from Regional Health Agency of Pays de la Loire. No support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years, no other relationships or activities that could appear to have influenced the submitted work

**Author Contributions**: Conceived and designed the experiments: JCR, DD, MK PYA. Analyzed the data: JCR DD MK PYA CYB. Wrote the paper: JCR DD PYA. Data acquisition: JCR, CF, JCP, CS, DM,
BB, US. Revision and critical review of the manuscript: JCR, DD, JCP, AL, OC, DM, US.

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Figure 1. Flow charts.
Figure 2. The K-ABC Mental Processing Composite score (mean, SD) at five years as a function of breastfeeding status at time of discharge and corrected age (*) at which infants were weaned off breastfeeding, in EPIPAGE cohort.‡ p adjusted for propensity score.
Figure 3. Weight, head circumference and height expressed as Z-score at discharge, 6 or 9 months and 2 years of corrected age (EPIPAGE and LIFT cohorts), and at 5 years (EPIPAGE cohort) according to breastfeeding at discharge. Weight measurements was known for 1460, 1430, 973, 873, 1447 infants at birth, discharge, 6 months, 2 and 5 years respectively in EPIPAGE, and for 1463, 1463, 1341 and 1297 infants at birth, discharge, 9 months and 2 years respectively in LIFT cohort.
January 11, 2012

RE: manuscript #2011.001225, entitled, “The apparent breastfeeding paradox in very preterm infants”: relationship between breastfeeding, early weight gain, and neurodevelopment based on results from two French cohorts.

Dear Dr Walker,

We appreciate your letter dated December 29, 2011, and the Reviewers’ constructive criticism. We have tried our best to accommodate their concerns in revising our manuscript, and submit the enclosed revised manuscript for hopeful publication in the BMJ Open.

Please find below our detailed answers to each of the Reviewers’ comments.

In the following, excerpts from the Reviewers comments are shown in Times New Roman italics, and immediately followed by our answers in Arial font.

We hope you will find our revision appropriate, and look forward to your editorial decision.

Yours sincerely,

Jean-Christophe Rozé, MD, PhD
Answers to Reviewer 1
# BMJ.2011.001225.R1 entitled “The Breastfeeding paradox in very preterm infants”

We appreciate Reviewer 1’s insightful comments. In the following, excerpts from the Reviewer’s comments are shown in italics, and immediately followed by our answers in Arial font.

Comments:
This is an interesting paper which draws attention to disparate effects of breastfeeding on the early ponderal growth of preterm infants and their subsequent neurological development. The analysis performed uses data from two large French preterm birth cohorts born six years apart. As the authors point out hospital nutritional practices may have changed over this interval, and possibly this explains differing relationships between growth in hospital and neurodevelopmental outcome observed in each. There could, however, be other explanations since different methods of neurodevelopmental assessment were applied at different ages.

Answer: We agree with the Reviewer’s comment. LIFT is an open, ongoing cohort: the population is followed until 5 years of age, and evaluation was complemented with an assessment of school performance by school teachers during the 6th year of life in kindergarten. Only a fraction of patients enrolled in LIFT cohort had reached 5 years when the study was performed. In the patients who reached 5 years, a very good correlation between 2-yrs ASQ and school performance as well as IQ was observed (unpublished data, manuscript in preparation). We therefore think the evaluation of neurodevelopment using ASQ is reliable.

The area that the authors have attempted to study is an extremely difficult one. It is important to appreciate that one is necessarily dependent on observational data in this field of enquiry since it would be unethical to allocate preterm infants at random to breastfeeding. In their discussion the authors clearly set out their appreciation of the biases and confounders inherent in this topic and Table 1 shows the large number of sociodemographic, neonatal phenotypic and treatment variables that are associated with breastfeeding at discharge. The authors have “adjusted” for these using a “breastfeeding propensity score”. This is not an approach I know but appropriate citations have been included. Despite these I do not think the nature of this score is explained sufficiently clearly for the reader as different contributors to the models applied are mentioned at various points in the manuscript. For example page 5 suggests that birthweight z-score and gestational age were used in the calculation of “propensity score” but at other points there is mention of adjusting for “propensity score”, gestational age and birthweight z-score together (e.g. pages 8 and 9). Confusingly birthweight z-score is also used to calculate in-hospital weight gain (as difference birth and discharge z-score) which is then “....adjusted for” [amongst other things] birthweight z-score and propensity score (Table 4).

Answer: We agree with the Reviewer’s comment, we clearly run a risk of “over adjustment”. After extensive discussion we nevertheless decided to calculate the risk of non optimal neurodevelopmental outcome, with adjustment for sex, gestational age and birthweight z-score together because neurodevelopment and weight gain are closely influenced by these 3 variables. As we were concerned about the risk of an under adjustment, and as recommended by D’Agostino (Statistics in Medicine, 1998, 17 : 2265-2281) we decided to adjust for variable closely associated with outcome, even though the variables are already included in the calculation of propensity score. This point is now specified (gestational age and birth weight Z-score Due to the known relationship between gestational age, weight gain and suboptimal...
development, these variables were included as confounding factors in the multivariable model even if they are already included in the calculation of propensity score, as suggested by other authors in the text in the revised manuscript (page 6, lines 12).

Nevertheless, we calculated adjusted OR with adjustment either (a) for propensity score only; or (b) for propensity score plus sex, gestational, age and birth weight Z-Score. We observed very similar aOR with both approaches:

<table>
<thead>
<tr>
<th>RISK OF NON OPTIMAL OUTCOME</th>
<th>OR adjusted for propensity score only</th>
<th>OR adjusted for propensity score, sex, birth weight Z-score, and GA</th>
</tr>
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<tbody>
<tr>
<td>In EPIPAGE cohort</td>
<td>OR = 0.65 (0.47-0.89)</td>
<td>OR = 0.65 (0.47-0.89)</td>
</tr>
<tr>
<td>In LIFT cohort</td>
<td>OR = 0.64 (0.45-0.88)</td>
<td>OR = 0.63 (0.45-0.87)</td>
</tr>
</tbody>
</table>

A couple of more minor points:

- I think the authors should be more specific in their use of the word “growth” when by and large they mean “weight gain”. Although it has widespread clinical currency as a day-to-day measure the latter is of course a poor descriptor of changes in body composition and metabolic function accompanying growth, let alone brain growth. When viewed from this perspective the authors may find their findings less of a “paradox”.

**Answer:** We replaced the term ‘growth’ with ‘weight gain’ throughout the manuscript when it was appropriate.

- The term “postconceptional age” (figure 2) is variously used and “corrected age” (which is I think what the authors mean) has gained more widespread acceptance (see Engle WA. Age terminology during the perinatal period. Pediatrics 2004;114:1362-4.)

**Answer:** We made the requested correction in the revised manuscript.

In summary, this is an interesting paper which makes a provocative point of much clinical relevance (viz. that weight gain is a poor predictor of developmental outcome in the breastfed preterm baby) but the statistical modelling employed is complex and requires close examination. The variables used in adjustment may not be entirely independent either of each other or of the outcome (in the case of weight gain). This could be more clearly explained to the reader.
Answer: We agree with reviewer's comment. We attenuated our provocative message in the title and in the first and second paragraph of discussion. We now explain the term of apparent paradox by this sentence: “Nevertheless, this paradox is probably only an apparent paradox because weight gain during hospitalization is a poor predictor of the quality of growth, as it does not provide any insight into the changes in body composition (page 8, line 31)

Similarly, we now state the limitations of the approach pointed out by Reviewer 1: “the variables used in adjustment may not be entirely independent either of each other or of the outcome” (page 10, line 29)
Answers to Reviewer 2

# BMJ.2011.001225.R1 entitled “The Breastfeeding paradox in very preterm infants”

We appreciate Reviewer 2’s insightful comments. In the following, excerpts from the Reviewer’s comments are shown in italics, and immediately followed by our answers in Arial font.

Comments:

The aims of the study were to assess the complex relationship of BF at discharge with growth and development. The investigators hypothesized that sub-optimal nutrition in NICU is associated with later poor cognitive function. The investigators studied the growth pattern of very preterm infants who were breast fed during their stay in the hospital (NICU) and then after discharge and assessed their development at 2-5 years of life. During the hospital stay breast milk feeding was supplemented with fortifiers but not after discharge. (This is one of the variables that needs close analysis How much, caloric density etc.) They compared the weight changes during and after discharge using Z scores. In addition they also measured the changes in head circumference.

Answer: The Reviewer is right, the information regarding how much fortifier was given would be helpful but is, unfortunately, not available in either database. This is addressed in the revised discussion (see ‘Weaknesses’ section, fifth paragraph of discussion): “Moreover, how much fortifier was given could, unfortunately not be retrieved from either of the two databases”. (page 10, line 31)

As stated in the manuscript, the loss of Z-score was less in the more recent LIFT cohort, suggesting a better use of fortifiers. Nevertheless, in each cohort, breastfeeding is associated with a greater loss of weight Z-score. This obviously is an association, and does not prove a causal relationship. However, we know for sure that fortification is most often discontinued as soon as suckling is felt / judged to be satisfactory, so as not to disturb suckling. This problem is at the very root of the current study.

MATERIAL AND METHODS:

Population included in this study was from two different studies from two different periods: The EPIPAGE study and the LIFT study. EPIPAGE study included infants born between 22 and 32 weeks of gestation in 1997 who survived (n=2282). For developmental assessment infants whose status regarding breastfeeding at time of discharge was known (n=2163) were included in this study. In this group the neuropsychological assessment was performed using the Kaufman Assessment Battery for Children (K-ABC) at five years of age. This scale is standardized to a mean of 100 (SD 15). An MPC in the lower tercile (score of less than 85) was considered as an index of suboptimal neurodevelopment.

In LIFT cohort 1733/1857 surviving babies with gestational age less than 33 weeks born between 2003 and 2008 those whose status regarding breastfeeding at time of discharge was known. In this Cohort, neuro-developmental assessment was performed using age and stages questionnaires (ASQ), a questionnaire completed by parents at a corrected age of two years. They compared growth pattern in the NICU with later developmental quotients in both groups. Propensity Score was calculated in each cohort to find the differences in both cohorts that may influence the growth pattern during the hospital stay and after discharge. Variables significantly associated with
Breastfeeding at discharge were very similar in both cohorts.

MAJOR FINDINGS.
In both cohorts, breastfeeding was associated with a significant reduction of risk for a suboptimal neurodevelopmental assessment at 2 years of corrected age (LIFT cohort) and at 5 Years, in EPiPAGE cohort.
Breastfeeding was associated with an increased risk of losing one weight Z-Score during NICU stay (Correction on line 46 in NOT in hospitalization)?

Answer: We agree. We made the correction

Growth during neonatal hospitalization, exposure to breastfeeding, and neurodevelopment This is the most important findings of the study. During hospitalization restricted growth (i.e., lower birth weight Z score) was associated before and after adjustment for gestational age and sex with later suboptimal neurodevelopmental assessment in both cohorts.

Page 9 line 15-21 the statement says :”After adjustment for weight Z Score at birth, sex and propensity score, breastfeeding at discharge was significantly associated to an increased risk of having a head circumference Z-score higher than 0.5 at five years in EPiPAGE cohort, (n=1412, aOR=1.47,[1.10-1.95]) and at two years of corrected age in LIFT cohort”
It is an advantage rather than a risk!

Answer: We agree: achieving a higher Z-score in head circumference obviously is an advantage rather than an unwanted change: the term ‘risk’ was simply used, as commonly used by statisticians, with no reference to the desirability of the change observed. We now replaced ‘risk’ with ‘chance’ in the revised manuscript (page 8, line 14).

STRENGTHS OF THE STUDY: The study includes large population data from two different cohorts at different times. Large proportion of population included in Follow up study. Good statistical analysis

Answer: We appreciate the comment.

WEAKNESSES:

1. Propensity score enables better comparison between the cohorts and groups however the calculation of PROPENSITY score did not include important variables that affect weight gain in the NICU e.g. time taken to start oral feeds, duration of hyper alimentation, lack of uniform protocols in regard to initiation of oral/tube feeding. Particularly there was no attempt to calculate the caloric intake during NICU stay

Answer: We agree. These variables were not collected in the database, and are now collected prospectively in the new, EPiPAGE II study, a prospective population-based cohort study in 25 regions of France in 2011, including 5000 very preterm children born alive. Associations between weight gain and neurodevelopmental outcome will be studied taking into account these parameters (age at the start of oral feeds, duration of intravenous nutrition, lack of uniform protocol,…). These
limitations are now stated in the, fifth paragraph of discussion in revised manuscript (‘Weaknesses’ section):

“Moreover, how much fortifier was given could, unfortunately, not retrieved from either of the two databases, and thus some important variables such as overall caloric intake during neonatal intensive care unit stay could not be included in the analysis.” (Page 10, Line 31)

2. It is an observational study hence associated deficiencies

Answer: We agree, and weaknesses are addressed in discussion.

3. No explanation for the mechanism. Although difficult to pin point authors should provide possible mechanisms for eg better bonding, better care given by parents

Answer: We agree, and now address this point in revised discussion, fifth paragraph mother-child interaction (better bonding, better care given by parents) (Page 11, Line 4)

There are a few minor typographical errors: page 10, line 47 LIKELY, Line 49 CAN

Answer: theses errors are corrected

COMMENTS

The main findings of the study are that in both independent cohorts of preterm infants studied, breastfeeding at discharge (continuation of breast feeding after discharge? ) was associated with a reduction in the risk for a suboptimal neurodevelopmental assessment at two or five years of age despite a higher risk for suboptimal growth (loss of one weight Z-Score) during neonatal hospitalization in these breastfed infants. They define these findings as “breastfeeding paradox” , meaning that breast feeding in the NICU was associated with poor weight gain but continuation of breast feeding after discharge was associated with better neurodevelopmental outcome at 2 and 5 years of age in very preterm infants. These findings are of critical importance and provide strong evidence for breast feeding in extreme premature infants both during NICU stay and after discharge. It is agreed that taken all data from literature and current study there is a consistence benefit of BF on later neuro development. And it provides evidence to encourage mothers who wish to breast feed, (Page 11 Lines 12-20 …)

However calling it a “Breastfeeding Paradox” is debatable. What is the paradox? Loss of weight by one Z score during hospital stay can be well explained independent of breast feeding. Suboptimal growth in extreme premature babies in the NICU is well recognized even in the formula fed infants. Such suboptimal growth is due to difficulties in delivering optimal calories to sick VLBW infants: illness, limitation of feeding, fluid intake and associated physiological constraints that affect nutritional intake in this population. However the beneficial effects of breast feeding on neurodevelopmental outcome are well recognized and the findings of this study confirm previous studies.

My argument against the use of the term “Breast Feeding Paradox” is that it implies that breast feeding in NICU is THE CAUSE for low birth weight z scores. There are several factors that influence the weight growth in THE NICU which may adversely influence the
growth of very preterm infants. As the investigators found that there was no association between NICU postnatal growth and developmental findings at 5 yrs. (p 8 line 55-60). Because nutrition was better in LIFT cohort. This again suggests that breast feeding is NOT the only cause low weight gain during the NICU stay. The study strengthens the concept that moms should be Advised to continue breast feeding of very premature babies after discharge from NICU.

Answer: We agree with Reviewer 2. First, the paradox may only be apparent. We therefore modified the title to ‘The apparent breast feeding paradox’ in the revised manuscript and we justify the term ‘apparent’ in the second paragraph of the discussion: “Nevertheless, this paradox is probably only an apparent paradox because weight gain during hospitalization is a poor predictor of the quality of growth, as it does not provide any insight into the changes in body composition”.

We also agree on the fact that the association between breastfeeding and loss of weight Z-score, does not prove causality, so breastfeeding may not be the only cause for the loss of weight Z-score in the NICU in the breast-fed infants. The association, however, is puzzling, as it was consistently found in both cohorts. Also, we believe a paradox exists since a better growth is generally associated with a better neurodevelopment in very preterm infants. In our study, we observe an association between breastfeeding and a better development, despite the loss of weight Z-score associated with breastfeeding. This indeed seems to be a paradox, since, even after adjusting for breastfeeding and propensity score, a loss of one Z-score is associated with suboptimal neurodevelopment in one cohort (LIFT cohort; adjusted OR: 1.28 (1.07-1.53); Table 5). This may, however, only be an apparent paradox, since weight gain a poor measurement of the quality of growth, as weight gain may not reflect fat free mass or brain growth. We therefore have initiated an ongoing study including assessment of body composition, and its relationship with neurodevelopment. We replace now ‘breastfeeding paradox’ by ‘apparent breastfeeding paradox’.
Table 1. Demographic and Clinical Characteristics of Very Low Gestational Age Infants Breastfed or Not at Time of Discharge.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EPIPAGE COHORT (EPIPAGE)</th>
<th>LIFT COHORT (LIFT)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristics of newborns</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age, w</td>
<td>30.4 (1.8)</td>
<td>29.8 (2.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>1460 (400)</td>
<td>1360 (390)</td>
<td>0.001</td>
</tr>
<tr>
<td>Birth weight, Z-Score</td>
<td>-0.23 (1.15)</td>
<td>-0.28 (1.05)</td>
<td>0.26</td>
</tr>
<tr>
<td>Male gender, n=409</td>
<td>212 (51.8)</td>
<td>952 (54.3)</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>Characteristics of mothers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age less than 25 years, n=409</td>
<td>49 (12.0)</td>
<td>372 (21.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>Two or more children at home, n=409</td>
<td>57 (13.9)</td>
<td>349 (19.9)</td>
<td>0.005</td>
</tr>
<tr>
<td>Single, n=409</td>
<td>18 (4.4)</td>
<td>157 (9.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Upper level education, n=409</td>
<td>178 (43.5)</td>
<td>430 (24.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>No professional activity, n=409</td>
<td>38 (9.3)</td>
<td>338 (19.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Health insurance for low financial income, n=409</td>
<td>-</td>
<td>12 (4.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Upper socio-demographic level, n=409</td>
<td>211 (51.6)</td>
<td>527 (30.0)</td>
<td>0.001</td>
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<tr>
<td><strong>Characteristics of pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension during pregnancy, n=409</td>
<td>99 (24.2)</td>
<td>381 (21.7)</td>
<td>0.276</td>
</tr>
<tr>
<td>Multiple pregnancy, n=409</td>
<td>101 (24.7)</td>
<td>572 (32.6)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Neonatal Hospitalization</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of mechanical ventilation, days, n=409</td>
<td>3.1 (7.5)</td>
<td>5.6 (11.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>Length of hospital stay, days, n=409</td>
<td>52.5 (24.1)</td>
<td>62.2 (32.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>Change in weight Z-score during neonatal hospitalization, n=409</td>
<td>-1.00 (0.63)</td>
<td>-0.95 (0.63)</td>
<td>0.170</td>
</tr>
<tr>
<td><strong>Follow-up &amp; Outcome</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lost to follow-up, n=409</td>
<td>49 (12.0)</td>
<td>361 (20.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>Follow-up, n=360</td>
<td>360 (88.0)</td>
<td>1393 (79.4)</td>
<td>0.738</td>
</tr>
<tr>
<td>Incomplete neuro-developmental evaluation, n=409</td>
<td>44 (12.2)</td>
<td>247 (17.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Normal neuro-development, n=409</td>
<td>252 (70.0)</td>
<td>725 (52.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Non Optimal neuro-development, n=409</td>
<td>64 (17.8)</td>
<td>421 (30.2)</td>
<td></td>
</tr>
</tbody>
</table>

Data are means (SD) or n (%). Non optimal neuro-development was defined as a KABC mental composite processing less than 85 at five years in EPIPAGE cohort and an ASQ score less than 220 at two years of corrected age in LIFT cohort.

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Table 2. Significant Associations between the Variables Included in Calculation of Propensity Score and Breast feeding.

<table>
<thead>
<tr>
<th>Characteristics of the mothers</th>
<th>EPIPAGE COHORT</th>
<th>LIFT COHORT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=2130</td>
<td>n=1733</td>
</tr>
<tr>
<td>Age less than 25 years</td>
<td>0.61 (0.42 - 0.89)</td>
<td>0.010</td>
</tr>
<tr>
<td>Upper socio-demographic level</td>
<td>1.77 (1.34 - 2.34)</td>
<td>0.001</td>
</tr>
<tr>
<td>Higher education</td>
<td>1.55 (1.17 - 2.06)</td>
<td>0.002</td>
</tr>
<tr>
<td>No Professional activity</td>
<td>0.66 (0.44 - 1.00)</td>
<td>0.052</td>
</tr>
<tr>
<td>Mother of foreign origin</td>
<td>1.50 (1.05 - 2.14)</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Characteristics of Pregnancy

| Multiple pregnancy            | 0.51 (0.39 - 0.67) | 0.001 | 0.69 (0.48 - 0.99) | 0.047 |

Characteristics of the newborns

| Duration of neonatal hospitalization (by week) | 0.89 (0.84 - 0.94) | 0.001 | 0.91 (0.85 - 0.97) | 0.004 |
| Gestational age (per week)                  | 1.03 (0.92 - 1.16) | 0.650 | 0.98 (0.86 - 1.10) | 0.714 |
| Birth weight (per Kg)                       | 1.17 (0.66 - 2.08) | 0.590 | 1.13 (0.70 - 1.83) | 0.612 |

*aOR, adjusted odd ratio
Table 3. Association between breast feeding at time of discharge and non optimal neuro-developmental performance.

<table>
<thead>
<tr>
<th>Adjustment</th>
<th>EPIPAGE Cohort n=1462</th>
<th>LIFT Cohort n=1463</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR 95%CI p</td>
<td>OR 95%CI p</td>
<td></td>
</tr>
<tr>
<td>No adjustment</td>
<td>0.44 (0.33 - 0.60) 0.001</td>
<td>0.53 (0.39-0.73) 0.001</td>
</tr>
<tr>
<td>Adjusted for gestational age, birth weight Z-score and sex.</td>
<td>0.46 (0.34 – 0.62) 0.001</td>
<td>0.57 (0.41-0.78) 0.001</td>
</tr>
<tr>
<td>Adjusted for gestational age, birth weight Z-score, sex and propensity score*</td>
<td>0.65 (0.47 – 0.89) 0.008</td>
<td>0.63 (0.45-0.87) 0.005</td>
</tr>
</tbody>
</table>

Non optimal neuro-development was defined as a KABC mental composite processing less than 85 at five years in EPIPAGE cohort and a ASQ score less than 220 at two years of corrected age in LIFT cohort.

*n=1443.
Table 4. Association between breast feeding at time of discharge and slow postnatal growth defined as a loss of one Z-score unit for weight during neonatal hospitalization.

<table>
<thead>
<tr>
<th>Adjustment</th>
<th>EPIPAGE Cohort</th>
<th>LIFT Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=1430</td>
<td>n=1463</td>
</tr>
<tr>
<td></td>
<td>OR  95%CI</td>
<td>P</td>
</tr>
<tr>
<td>No adjustment</td>
<td>1.12 (0.87 - 1.43)</td>
<td>0.38</td>
</tr>
<tr>
<td>Adjusted for gestational age, sex, birth weight Z-score</td>
<td>1.32 (0.98 - 1.76)</td>
<td>0.07</td>
</tr>
<tr>
<td>Adjusted for gestational age, sex, birth weight Z-score and propensity score*</td>
<td>1.55 (1.14 - 2.12)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

* n = 1417.
<table>
<thead>
<tr>
<th></th>
<th>EPIPAGE Cohort</th>
<th>LIFT Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95%CI p</td>
<td>OR 95%CI</td>
</tr>
<tr>
<td>Birth weight (per one Z-score unit below the mean)</td>
<td>n=1460</td>
<td>n=1463</td>
</tr>
<tr>
<td>No adjustment</td>
<td>1.19 (1.04-1.35)</td>
<td>0.01</td>
</tr>
<tr>
<td>Adjusted for gestational age, sex</td>
<td>1.23 (1.07-1.40)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

| Postnatal Growth for weight (per one Z-score unit lost between birth and discharge) | n=1430 | n=1463 |
| No adjustment          | 0.93 (0.78 - 1.11) | 0.44        | 1.29 (1.10-1.51) | 0.002     |
| Adjusted for gestational age, sex, birth weight Z-score | 0.97 (0.79 – 1.19) | 0.74       | 1.21 (1.01-1.44) | 0.038     |
| Adjusted for gestational age, sex, birth weight Z-score, breastfeeding and propensity score * | 1.06 (0.85 – 1.31) | 0.63       | 1.28 (1.07-1.53) | 0.008     |

Non optimal neuro-development was defined as a KABC mental composite processing less than 85 at five years in EPIPAGE cohort and a ASQ score less than 220 at two years of corrected age in LIFT cohort.

* n = 1417
Flow charts

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The K-ABC Mental Processing Composite score (mean, SD) at five years as a function of breastfeeding status at time of discharge and corrected age (*) at which infants were weaned off breastfeeding, in EPipe cohort.

‡ p adjusted for propensity score.

122x91mm (600 x 600 DPI)
Weight, head circumference and height expressed as Z-score at discharge, 6 or 9 months and 2 years of corrected age (EPIPAGE and LIFT cohorts), and at 5 years (EPIPAGE cohort) according to breastfeeding at discharge. Weight measurements was known for 1460, 1430, 973, 873, 1447 infants at birth, discharge, 6 months, 2 and 5 years respectively in EPIPAGE, and for 1463, 1463, 1341 and 1297 infants at birth, discharge, 9 months and 2 years respectively in LIFT cohort.
<table>
<thead>
<tr>
<th>Item No</th>
<th>Recommendation</th>
<th>Title and abstract</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(a) Indicate the study’s design with a commonly used term in the title or the abstract</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>(b) Provide in the abstract an informative and balanced summary of what was done and what was found</td>
<td>2</td>
<td></td>
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<tr>
<td>2</td>
<td>Explain the scientific background and rationale for the investigation being reported</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>State specific objectives, including any prespecified hypotheses</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Present key elements of study design early in the paper</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>(b) For matched studies, give matching criteria and number of exposed and unexposed</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>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</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Describe any efforts to address potential sources of bias</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Explain how the study size was arrived at</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>(a) Describe all statistical methods, including those used to control for confounding</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>(b) Describe any methods used to examine subgroups and interactions</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>(c) Explain how missing data were addressed</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>(d) If applicable, explain how loss to follow-up was addressed</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>(e) Describe any sensitivity analyses</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>(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</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>(b) Give reasons for non-participation at each stage</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>(c) Consider use of a flow diagram</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>(b) Indicate number of participants with missing data for each variable of interest</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>(c) Summarise follow-up time (eg, average and total amount)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Report numbers of outcome events or summary measures over time</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>(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</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>(b) Report category boundaries when continuous variables were categorized</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses</td>
<td>7</td>
<td></td>
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</table>
### Discussion

<table>
<thead>
<tr>
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<th>Page</th>
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<tbody>
<tr>
<td>Key results</td>
<td>18</td>
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<tr>
<td>Summarise key results with reference to study objectives</td>
<td>8</td>
</tr>
<tr>
<td>Limitations</td>
<td>19</td>
</tr>
<tr>
<td>Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias</td>
<td>10</td>
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<tr>
<td>Interpretation</td>
<td>20</td>
</tr>
<tr>
<td>Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence</td>
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<tr>
<td>Generalisability</td>
<td>21</td>
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<tr>
<td>Discuss the generalisability (external validity) of the study results</td>
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<tr>
<td>Other information</td>
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</tr>
<tr>
<td>Funding</td>
<td>22</td>
</tr>
<tr>
<td>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</td>
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The apparent breastfeeding paradox in very preterm infants: relationship between breastfeeding, early weight gain, and neurodevelopment based on results from two cohorts, EPIPAGE and LIFT.

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<td>29-Feb-2012</td>
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<td>Complete List of Authors:</td>
<td>ROZE, JEAN-CHRISTOPHE; INSERM, CIC 004 Darmaun, Dominique; INRA; Nantes University, UMR 1280 PhAN Boquien, Clair-Yves; INRA; Nantes University, UMR 1280 PhAN Flamant, Flamant; CHU de Nantes, Department of Neonatology Ricaud, Jean-Charles; Hopital de la Croix-Rousse, Department of Neonatal Medicine Savagner, Christophe; CHU d'Angers, Department of Neonatal Medicine Claris, Olivier; Hôpital Edouard Herriot, Department of Neonatal Medicine Lapillonne, Alexandre; Paris Descartes University, Assistance Publique-Hôpitaux de Paris, Necker hospital, Department of Neonatal Medicine Mitanchez, Delphine; Assistance Publique-Hôpitaux de Paris, Réanimation Néonatale et Pédiatrique, Hôpital Trousseau, Department of Neonatal Medicine Branger, Bernard; “Loire Infant Follow-up Team” (LIFT) Network, Pays de Loire, Simoeni, Umberto; Neonatal Intensive Care Unit, La Conception University Hospital, Department of Neonatal Medicine Kaminski, Monique; INSERM, Hôpital Tenon, UMR 953, Epidemiological Research in Perinatal Health and Women's and Children Health, Ancel, Pierre-Yves; INSERM, Hôpital Tenon, UMR 953, Epidemiological Research in Perinatal Health and Women's and Children Health,</td>
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The apparent breastfeeding paradox in very preterm infants: relationship between breastfeeding, early weight gain, and neurodevelopment based on results from two cohorts, EPISODE and LIFT.

Jean-Christophe Rozé,1,2,3,4 MD, PhD, Dominique Darmaun4, MD, PhD, Clair-Yves Boquien4, PhD, Cyril Flamant,1,2,3, MD, PhD, Jean Charles Picaud,5 MD, PhD, Christophe Savagner, MD,6 Olivier Claris,7 MD, , Alexandre Lapillonne,8 MD, PhD; Delphine Mitanche,9 MD, PhD; Bernard Branger,3 MD; Umberto Simeoni,10 MD, PhD, Monique Kaminski,11 MSc,PhD; Pierre-Yves Ancel,11 MD, PhD.

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ABSTRACT

CONTEXT: Supplementation of breast milk is difficult once infants suckle the breast and is often discontinued at end of hospitalization and after discharge. Thus breastfed preterm infants are exposed to an increased risk of nutritional deficit with a possible consequence on neurodevelopmental outcome.

OBJECTIVE: To assess the relationship between breastfeeding at time of discharge, weight gain during hospitalization and neurodevelopmental outcome.

DESIGN: Observational cohort study.

SETTING: Two large independent population based cohorts of very preterm infants: the LIFT and the EPIPAGE cohorts.

PATIENTS: 2925 very preterm infants alive at discharge.

MAIN OUTCOME MEASURE: Suboptimal neurodevelopmental outcome, defined as a score in the lower tercile, using Age and Stages Questionnaire at 2 years in LIFT and K-ABC test at 5 years in EPIPAGE. Two propensity scores for breastfeeding at discharge, one for each cohort, were used to reduce bias.

RESULTS: Breastfeeding at time of discharge concerned only 278/1733 (16%) in LIFT and 409/2163 (19%) infants in EPIPAGE cohort. Breastfeeding is significantly associated with an increased risk of losing one weight Z-Score during hospitalization (LIFT: n=1463, aOR=2.51 [1.87-3.36]; EPIPAGE: n=1417, aOR=1.55 [1.14-2.12]) and with a decreased risk for a suboptimal neurodevelopmental assessment (LIFT: n=1463, aOR=0.63 [0.45-0.87]; EPIPAGE: n=1441, aOR=0.65 [0.47-0.89] and an increased chance of having a head circumference Z-score higher than 0.5 at two years in LIFT cohort. (n=1276, aOR=1.43, [1.02-2.02]) and at five years in EPIPAGE cohort, (n=1412, aOR=1.47, [1.10-1.95]).

CONCLUSION: The observed better neurodevelopment in spite of suboptimal initial weight gain could be termed the ‘apparent breastfeeding paradox’ in very preterm infants. Regardless of the mechanisms involved the current data provide encouragement for the use of breastfeeding in preterm infants.
INTRODUCTION

Breastfeeding is universally recommended for the feeding of term infants (1). Regarding very preterm infants, less than 32 weeks of gestation, exclusive breastfeeding is a debated topic since supplementation is required to ensure optimal growth during initial hospitalization but is difficult once the preterm infant can suckle the breast (2).

Suboptimal nutrition with insufficient growth during hospitalization in neonatal intensive care unit is associated with later cognitive dysfunction (3,4). In extremely preterm infants, growth velocity during hospitalization exerts a significant, and possibly independent, effect on neurodevelopment and growth outcomes at 18 to 22 months’ corrected age (3). In preterm infants, follow up studies showed that at 7 years of age, preterm infants fed standard formula demonstrated neurocognitive impairment with a significant reduction in IQ, compared with infants fed with enriched formula(4).

Breastfeeding with supplementation during initial hospitalization improves cognitive outcome at 30 months of corrected age in extremely preterm infants (5). Human milk indeed requires nutrient fortification to meet the protein and mineral needs of the rapidly growing preterm infant (6). During hospitalization, as the baby receives mother-milk through a gastric tube it is easy to use a milk fortifier to maintain adequate growth. Once the preterm infant can suckle the breast, however, the use of a milk fortifier is not easy, as it disrupts the routine of breastfeeding. As a consequence, mother milk supplementation is often discontinued at the end of hospitalization and hospital discharge, and this discontinuation exposes the infants to an increased risk of nutritional deficit (7). Thus, breastfeeding at time of discharge could be associated with less weight gain during neonatal hospitalization and during the weeks following discharge. This is why exclusive breastfeeding remains a matter of debate in preterm infants.

The aim of the current study was to assess the complex relationship between breastfeeding at time of discharge, weight gain during neonatal hospitalization and neurodevelopment at 2 or 5 years using data from two independent large cohorts of very preterm infants of less than 33 weeks’ gestation covering the late 1990s and mid-2000s. The secondary objective was assessment of growth (weight, height, head circumference) at 2 and 5 years.
Methods

Data Source and Patients:

Date source is constituted of two cohorts: EPIPAGE (1) and LIFT (Loire Infant Follow-up Team) (8) cohorts with recruitment over two distinct periods. EPIPAGE is a prospective population-based cohort study including all infants born between 22 and 32 weeks of gestation in 1997 in the maternity wards of nine French regions accounting for about one third of all births in France. Among infants born in 1997, who survived and eligible for the follow up (n=2282), we included all infants whose status regarding breastfeeding at time of discharge was known (n=2163). LIFT cohort is a cohort of infants born in one region (Pays de la Loire, a region in Western France) and enrolled in the regional follow-up network. Among surviving very preterm infants with a gestational age less than 33 weeks of gestation, born between January 1, 2003 and June 30, 2008 and enrolled in LIFT cohort (n=1857), we included all children whose status regarding breastfeeding at time of discharge was known (n=1733) (Fig 1). Each cohort was registered to the French CNIL. For EPIPAGE cohort, parents were told about the study and given written information in the maternity or neonatal unit, and verbal consent was provided to the medical team in charge of the study at recruitment. For LIFT cohort, a written consent was obtained at enrollment.

Developmental assessment

In the EPIPAGE cohort, the neuropsychological assessment was performed using the Kaufman Assessment Battery for Children (K-ABC) at five years of age (9). Neuropsychological assessment was performed by trained psychologists when appropriate for the patient’s condition and when accepted by the patient. The K-ABC yields four global test scores. The Mental Processing Composite scale, which is considered to be equivalent to intelligence quotient (IQ), is a global measure of cognitive ability. This scale is standardized to a mean of 100 (SD 15). An MPC in the lower tercile (score of less than 85) was considered as an index of suboptimal neurodevelopment.

In the LIFT cohort, neuro-developmental assessment was performed using Age and stages questionnaires (ASQ) (10), a questionnaire completed by parents at a corrected age of two years. This questionnaire includes five parts, among which three are related to cognitive development: communication, problem solving, and personal social interactions. The sum of the 5 partial scores was calculated, and subject population was split in 3 terciles of global ASQ.
score. Being in the lower tercile (ASQ score less than 220) was considered an index of suboptimal neurodevelopment.

Growth assessment
To assess growth, we used measurements performed at birth, discharge, and 6 months, two years and 5 years of age in EPIPAGE cohort, birth, discharge, 9 months and 2 years in LIFT cohort. We calculated Z-Score by using LMS method. We used reference growth curves for which LMS parameters have been published for weight, height and head circumference. For birth and discharge (up to 41 weeks of postmenstrual age) measurements, we used Olsen’s preterm infant growth chart (11). For the few preterm infants discharged after 41 weeks of postmenstrual age, and for follow-up period measurements, we used World Health Organization (WHO) growth curves (12). Weight gain during hospitalization was assessed as the difference of weight Z-score between discharge and birth.

Statistical Analysis
Means and standard deviations are reported for continuous variables and frequencies for categorical variables. Anova and $\chi^2$ or Fisher test if necessary, were used to compare infant characteristics and 5-year outcome between the groups of infants who were breast fed or formula fed at time of discharge.

The propensity score method was used to reduce bias in assessing the relation between breastfeeding at discharge and cognitive outcomes (13-14). The propensity score is defined as a conditional probability, between 0 and 1, that a subject will be “breastfed at discharge” based on an observed group of covariates. This score is then used just as if it were the only confounding covariate. Thus, the collection of predictors is collapsed into a single predictor, which may better adjust covariates between the groups and reduce bias. Two full non-parsimonious logistic regression models were developed to derive a propensity score for breast feeding at discharge, one for each cohort. These models included true confounders: variables that are potentially associated with both mode of feeding and with outcome (15,16). These variables included for the Epipage cohort: characteristics of the mothers (age, maternal body mass index (17), level of maternal education, socioeconomic status, number of children at home), characteristics of the newborns (gestational age, Z-Score of birth weight, presence of a congenital malformation), characteristics of pregnancy (antenatal corticosteroids, multiple pregnancy, complication of pregnancy), place of birth (inborn/outborn, region of birth),
characteristics of neonatal hospitalization (cranial ultrasound abnormalities, patent ductus arteriosus, necrotizing enterocolitis, neonatal surgery, bronchopulmonary dysplasia defined by supplemental oxygen requirement at 36 weeks, duration of mechanical ventilation, length of hospital stay). Patients with missing data were excluded from multivariable analysis. For the LIFT cohort, the variables included in propensity score calculation were similar but less numerous, including characteristics of the mother, characteristics of the newborn and neonatal hospitalization. The Hosmer-Lemeshow goodness-of-fit test and the area under the curve were used to assess each model fit.

First, we studied crude associations between breastfeeding at discharge and suboptimal neurodevelopment assessment and then the same associations after adjustment for the propensity score, gestational age and birth weight Z-score. Due to the known relationship between gestational age, weight gain and suboptimal development, these variables were included as confounding factors in the multivariable model even if they are already included in the calculation of propensity score, as suggested by other authors (14). We used logistic regression models for univariate and multivariate analyses (16). We estimated the crude and adjusted odds ratio, and its 95% confidence interval, of a suboptimal neurodevelopmental score, i.e. to be in the lower tercile of MPC (less than 85) at 5 years in EPIPAGE cohort or ASQ (less than 220) at 2 years corrected age in LIFT cohort, associated with breastfeeding at discharge.

Secondly, we studied crude associations between breastfeeding and postnatal weight gain during neonatal hospitalization and thereafter, up to 2 years in LIFT cohort and 5 years in EPIPAGE cohort. Finally we studied the relationship between weight gain during neonatal hospitalization and suboptimal neurodevelopment assessment. Moreover, we assessed growth (weight, height, head circumference) at 6 months, 2 and 5 years in EPIPAGE and at 9 months, and two years in LIFT cohort. A supplementary propensity-score matching analysis was performed comparing matched pairs of breastfed and not-breastfed very preterm infants in each cohort. Matched pairs were created by their propensity score, and the outcomes of the two groups were compared in each cohort. All p values were based on two-sided tests. All analyses were performed using SPSS 15.0 (SPSS INC, Chicago, IL).

Results

Patients Characteristics.

The populations enrolled in EPIPAGE (n = 2163) and LIFT cohorts (n = 1733) were very similar with a small albeit significant difference in gestational age (29.9 ± 2.0 vs. 29.8 ± 2.1 weeks,
p=0.01), birth weight (1380 ± 395 vs. 1340 ± 396 g, p=0.01) postnatal weight gain (0.98. ± 0.79 vs. 0.83 ± 0.82 loss of weight Z-score, p=0.001) but not in length of hospital stay (60.2 ± 31.7 vs. 58.7 ± 32.2 days, p=0.13). The proportion of breastfed infants was a little bit higher in EPIPAGE cohort (19%) than in LIFT cohort (16%), p=0.02. In both cohorts breastfeeding was associated with some characteristics of the mothers, pregnancy, newborns and neonatal hospitalization (Table 1). Among the 2163 children of EPIPAGE cohort, 1753 (81%) were followed up to 5 years and 1462 (68%) assessed by trained psychologists. Among the 1733 of LIFT cohort, 1587 (85%) were followed up to two years of corrected age and we obtained ASQ for 1463 children (79%).

**Propensity Score** was calculated in each cohort. In EpiPage cohort, it was possible to calculate the propensity score for 2130 of the 2163 very preterm infants who were alive at hospital discharge and whose status regarding breast feeding status at time of discharge was known. The propensity scores ranged from 0.0005 to 0.722. The Hosmer-Lemeshow test was 7.2, p=0.51. The ROC curve area was 0.72 ± 0.01. In LIFT cohort, the propensity score was calculated for all preterm infants. For each variable, if necessary, a subgroup with unknown data was constituted. The propensity scores ranged from 0.007 to 0.747. The Hosmer-Lemeshow test was 6.2, p=0.63. The ROC curve area was 0.71 ± 0.01. Variables significantly associated with breastfeeding at discharge were very similar in both cohorts (Table 2).

**Exposure to Breastfeeding at Time of Discharge and neurodevelopmental Outcome**

In both cohorts, breastfeeding was associated with a significant reduction of risk for a suboptimal neurodevelopmental assessment at 2 years of corrected age (LIFT cohort) and at 5 years, (Table 3). Moreover, The K-ABC Mental Processing Composite score increased as a function of the corrected age at which infants were weaned off breastfeeding (Fig 2). In each cohort, breastfeeding was consistently associated with a reduction in the risk for suboptimal neurodevelopmental assessment before and after adjustment for gestational age, birth weight Z-score and sex, and propensity score (Table 3). *We observed the same results in propensity-score matching analysis (Supplemental table).*

**Weight gain during neonatal hospitalization and exposure to breastfeeding.**

Breastfeeding was associated with an increased risk of losing one weight Z-Score during hospitalization, before adjustment in LIFT cohort, and after adjustment in both cohorts (Table 4). The loss in weight Z-Score during NICU hospitalization was similar in both cohorts in breastfed infants but not in non breastfed infants: the loss was less pronounced in the most recent cohort.
(Table 1). We observed a greater loss in weight Z-Score in breastfed group in propensity-score matching analysis, and this for each cohort (Supplemental table).

Weight gain during neonatal hospitalization, exposure to breastfeeding, and neurodevelopmental outcome.

Restricted intrauterine growth (i.e., lower birth weight Z Score) was associated before and after adjustment for gestational age and sex with a suboptimal neurodevelopmental assessment in both cohorts. Postnatal weight gain during neonatal hospitalization was significantly associated with a suboptimal neurodevelopmental assessment only in LIFT cohort but not in in EPIPAGE cohort (Table 5), with and without adjustment for gestational age, birth weight Z-score, sex, breastfeeding and propensity score.

Growth after neonatal hospitalization.

In contrast with data on initial growth, from 2 years of corrected age, weight, height and head circumference were significantly higher in preterm infants who had been breastfed at the time of discharge in both LIFT and Epipage cohorts (Fig 2). After adjustment for weight Z Score at birth, sex and propensity score, breastfeeding at discharge was significantly associated to an increased chance of having a head circumference Z-score higher than 0.5 at five years in EPIPAGE cohort, (n=1412, aOR=1.47, [1.10-1.95]) and at two years of corrected age in LIFT cohort, (n=1276, aOR=1.43, [1.02-2.02]).

DISCUSSION

In two independent cohorts of preterm infants, breastfeeding at discharge was associated with a reduction in the risk for a suboptimal neurodevelopmental assessment at two (LIFT cohort) or five years of age (EPIPAGE cohort) despite a higher risk for suboptimal weight gain (loss of one weight Z-Score) during neonatal hospitalization in these breastfed infants. The observed improved neurodevelopment in spite of suboptimal initial weight gain could be termed the ‘apparent breastfeeding paradox’ in very preterm infants.

Such an observation is indeed a paradox as several earlier studies have documented an association between suboptimal early postnatal nutrition with insufficient weight gain during hospital stay and later cognitive dysfunction. In extremely preterm infants, faster weight gain in NICU was associated with improved outcome in terms of neurodevelopment and growth at 18 to
22 months’ corrected age. (3) Nevertheless, this paradox is probably only an apparent paradox
because weight gain during hospitalization is a poor predictor of the quality of growth, as it does
not provide any insight into the changes in body composition. In infants with a very low birth
weight, enhanced postnatal growth is also associated with a better later neurodevelopmental
outcome (18), especially regarding postnatal growth in head circumference, an index of brain
growth (19, 20). Yet the “neuroprotective” effects of improved growth may only be mild and
mainly concern growth over the first few weeks of hospital stay (21). In the two cohorts used in
the current analysis, the role of postnatal weight gain was slightly different. In the EPIPAGE
cohort, we did not observe any association between postnatal weight gain during hospitalization
and sub-optimal neurodevelopmental score at five years of age (Table 5). In the LIFT cohort, 
after adjustment for propensity score, a significant association was found between initial weight
gain rate and neurodevelopment. In contrast, in both cohorts a negative association was
obvious between birthweight Z-score and suboptimal neuro-development (Table 5). Such
difference between the 2 cohorts could be in relation with the significantly better initial neonatal
weight gain rate in the LIFT cohort, compared with the EPIPAGE cohort, as a matter of fact, the
loss in weight Z-score was less in the most recent cohort, presumably due to improvement in
care routines between the two periods (1997 vs. 2003) in the NICUs in France (22). Thanks to
the improvement in nutritional management in the second period, the role of postnatal nutrition,
adjusted for breastfeeding, may have become detectable in the more recent LIFT cohort.

Beneficial effects of breastfeeding on cognitive skills and behavioral scores have been
demonstrated previously in term (23, 24), preterm (25), and extremely preterm infants (5).
Multiple biases may, however, interfere, particularly maternal socioeconomic and educational
status: in the two cohorts, upper social status was obviously associated with a higher chance for
being breastfed at discharge, and for an optimal neurodevelopmental score. In term infants,
after control for biases (26)—particularly using the method of sibling comparison which
automatically controls for any confounding factors that are the same for each of the siblings in a
pair (27)—the role of breastfeeding has been found to be either not significant or modest. In
preterm infants, observational studies have shown that preterm infants whose mothers chose to
breastfeed reached a higher intelligence quotient at 8 years of age. Interestingly, infants whose
mothers were willing to breastfeed but failed to do so had the same IQ as those whose mothers
elected not to breastfeed (25). In preterm infants, observational studies found feeding with
human milk to be associated with a better outcome (5). Eight randomized studies were included
in a Cochrane review (28). Only 2 old randomized studies using unfortified donor human milk
reported a comparison of developmental outcome between unfortified human milk and formula
feeding. Follow-up of the infants who participated in these two trials did not find a significant
effect on long-term growth parameters or neurodevelopmental outcomes. Most of other
parameters reported by the randomized studies comparing formula to human milk were in favor
of breastmilk, except for weight gain during hospitalization. Moreover, among children who had
been enrolled in one of such studies Lucas et al observed 15 years later, that breastmilk
consumption was associated with a lower blood pressure in children born prematurely,
suggesting a long-term beneficial effects of breastmilk in preterm infants (29). Taken together,
data from the literature, and from the current study are consistent with a benefit of breastmilk
consumption on developmental outcome.

At the time of discharge, very preterm infants have accumulated deficits in energy, protein and
minerals, and, due to their early discharge, still have higher nutrient requirements than healthy
appropriate-for-gestational age term infants. Despite the vast body of published
recommendations on the nutrition of preterm infants, there is little data on optimal nutritional
management after discharge of breastfed preterm infants (30). No randomized studies can be
ethically performed to determine whether feeding preterm infants following hospital discharge
with nutrient-enriched formula milk versus human breast milk affects growth and development.
The conclusion of a Cochrane review (2) is that “mothers who wish to breast feed, and their
health care advisors, would require very clear evidence that feeding with a nutrient-enriched
formula milk had major advantages for their infants before electing not to feed (or to reduce
feeding) with maternal breast milk, and that evidence from trials that compared feeding preterm
infants following hospital discharge with nutrient-enriched versus standard formula milk
demonstrating an effect on growth or development “. The need to contribute an answer to the
nagging, disturbing question, ‘Does exclusive breastfeeding at the time of discharge influence
outcome?’ was the very incentive to perform the current study. Our analysis of two large cohorts
of preterm infant with relatively long term follow-up, used the propensity score method as a
means to control as much as possible for potential confounders. From such analysis we
obtained clear-cut evidence that despite an increased risk for suboptimal early weight gain
(increased risk for the loss of one weight Z-Score) during neonatal hospitalization, breastfeeding
at discharge is associated with a better outcome after adjustment for potential confounders by
using propensity score.

The main weakness of our study is the observational design. Despite the use of propensity
score we cannot ensure all potential confounders were eliminated. For instance, the very ability
of an infant to suckle may be associated with a less sick infant, and the variables used in
adjustment may not be entirely independent either of each other or of the outcome. Moreover,
how much fortifier was given could not, unfortunately, be retrieved from either of the two
databases, and thus some important variable such as caloric intake during neonatal intensive care unit stay could not be included in the analysis. Similarly, we did not address the mechanisms. For instance, the effect of breastfeeding for term infants has been attributed to bias (31), including the complex relationship between weight gain and timing of weaning. Such bias is unlikely to be relevant for our population since the preterm infants with the slower weight gain rate were those who were not weaned at discharge. The slower weight gain during neonatal intensive care unit stay therefore likely reflects a biological effect rather than bias.

Several potential mechanisms can be proposed to explain the better outcome in breastfed infants: mother-child interaction (better bonding, better care given by parents), the effect of specific nutrients contained in breastmilk such as polyunsaturated fatty acids, prebiotic oligosaccharides, etc. Regardless of mechanisms we did observe the same fact in two distinct cohorts, with the same magnitude. By adjustment for propensity score, sex, and birth weight Z-score, we were indeed able to observe the apparent paradox of a better neurodevelopmental outcome, despite a lower early weight gain. These adjustments are necessary because a complex relationship exists between breastfeeding, birth weight, gestational age, birth weight Z-score, and postnatal weight gain during hospitalization.

In conclusion, the neurodevelopment of premature infants is likely to benefit from feeding supplemented mother milk during hospital stay and unsupplemented mother’s milk after discharge, and these data from two cohorts are indeed reassuring, because in spite of a lesser weight gain during hospitalization, we observed a better neurodevelopmental outcome in the breastfed groups. The present report suggests breastfeeding should be recommended at the time of discharge. As the rate of exclusive breastfeeding at time of discharge is low—less than 30% in Europe (32)—strategies to facilitate breastfeeding at discharge must be developed (33). Moreover, when adjusted on breastfeeding, postnatal weight gain has a positive effect on neurodevelopmental outcome as observed in LIFT cohort; so the question about the putative benefit of human milk supplementation after discharge remains open. **Supplementation must be continued as long as possible, according to the state of knowledge and this suggests also that more research is warranted about human milk composition and the potential benefit of human milk supplementation at time of discharge is warranted in the future.**
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Author Contributions: Conceived and designed the experiments: JCR, DD, MK PYA. Analyzed the data: JCR DD MK PYA CYB. Wrote the paper: JCR DD PYA. Data acquisition: JCR, CF, JCP, CS, DM, BB,US. Revision and critical review of the manuscript: JCR, DD, JCP, AL, OC, DM, US, CB, FF, CS, BD, MK, PA.

Data Sharing: Data set is available by emailing PY Ancel pierreyves.ancel@inserm.fr for EPIPAGE cohort and jcroze@chu-nantes.fr for LIFT cohort.

Competing Interests: None.
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The apparent breastfeeding paradox in very preterm infants: relationship between breastfeeding, early weight gain, and neurodevelopment based on results from two cohorts, EPIPAGE and LIFT.

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**Statistical Analysis**

Means and standard deviations are reported for continuous variables and frequencies for categorical variables. Anova and χ² or Fisher test if necessary, were used to compare infant characteristics and 5-year outcome between the groups of infants who were breast fed or formula fed at time of discharge.

The propensity score method was used to reduce bias in assessing the relation between breastfeeding at discharge and cognitive outcomes (13-14). The propensity score is defined as a conditional probability, between 0 and 1, that a subject will be “breastfed at discharge” based on an observed group of covariates. This score is then used just as if it were the only confounding covariate. Thus, the collection of predictors is collapsed into a single predictor, which may better adjust covariates between the groups and reduce bias. Two full non-parsimonious logistic regression models were developed to derive a propensity score for breast feeding at discharge, one for each cohort. These models included true confounders: variables that are potentially associated with both mode of feeding and with outcome (15,16). These variables included for the Epipage cohort: characteristics of the mothers (age, maternal body mass index (17), level of maternal education, socioeconomic status, number of children at home), characteristics of the newborns (gestational age, Z-Score of birth weight, presence of a congenital malformation), characteristics of pregnancy (antenatal corticosteroids, multiple pregnancy, complication of pregnancy), place of birth (inborn/outborn, region of birth),
characteristics of neonatal hospitalization (cranial ultrasound abnormalities, patent ductus arteriosus, necrotizing enterocolitis, neonatal surgery, bronchopulmonary dysplasia defined by supplemental oxygen requirement at 36 weeks, duration of mechanical ventilation, length of hospital stay). Patients with missing data were excluded from multivariable analysis. For the LIFT cohort, the variables included in propensity score calculation were similar but less numerous, including characteristics of the mother, characteristics of the newborn and neonatal hospitalization. The Hosmer-Lemeshow goodness-of-fit test and the area under the curve were used to assess each model fit.

First, we studied crude associations between breastfeeding at discharge and suboptimal neurodevelopment assessment and then the same associations after adjustment for the propensity score, gestational age and birth weight Z-score. Due to the known relationship between gestational age, weight gain and suboptimal development, these variables were included as confounding factors in the multivariable model even if they are already included in the calculation of propensity score, as suggested by other authors(14). We used logistic regression models for univariate and multivariate analyses (16). We estimated the crude and adjusted odds ratio, and its 95% confidence interval, of a suboptimal neurodevelopmental score, i.e. to be in the lower tercile of MPC (less than 85) at 5 years in EPIPAGE cohort or ASQ (less than 220) at 2 years corrected age in LIFT cohort, associated with breastfeeding at discharge.

Secondly, we studied crude associations between breastfeeding and postnatal weight gain during neonatal hospitalization and thereafter, up to 2 years in LIFT cohort and 5 years in EPIPAGE cohort. Finally we studied the relationship between weight gain during neonatal hospitalization and suboptimal neurodevelopment assessment. Moreover, we assessed growth (weight, height, head circumference) at 6 months, 2 and 5 years in EPIPAGE and at 9 months, and two years in LIFT cohort. A supplementary propensity–score matching analysis was performed comparing matched pairs of breastfed and not-breastfed very preterm infants in each cohort. Matched pairs were created by their propensity score, and the outcomes of the two groups were compared in each cohort. All p values were based on two-sided tests. All analyses were performed using SPSS 15.0 (SPSS INC, Chicago, IL).

Results

Patients Characteristics.

The populations enrolled in EPIPAGE (n =2163) and LIFT cohorts (n=1733) were very similar with a small albeit significant difference in gestational age (29.9 ± 2.0 vs. 29.8 ± 2.1 weeks,
p=0.01), birth weight (1380 ± 395 vs. 1340 ± 396 g, p=0.01) postnatal weight gain (0.98 ± 0.79 vs. 0.83 ± 0.82 loss of weight Z-score, p=0.001) but not in length of hospital stay (60.2 ± 31.7 vs. 58.7 ± 32.2 days, p=0.13). The proportion of breastfed infants was a little bit higher in EPIPAGE cohort (19%) than in LIFT cohort (16%), p=0.02. In both cohorts breastfeeding was associated with some characteristics of the mothers, pregnancy, newborns and neonatal hospitalization (Table 1). Among the 2163 children of EPIPAGE cohort, 1753 (81%) were followed up to 5 years and 1462 (68%) assessed by trained psychologists. Among the 1733 of LIFT cohort, 1587 (85%) were followed up to two years of corrected age and we obtained ASQ for 1463 children (79%).

**Propensity Score** was calculated in each cohort. In Epipage cohort, it was possible to calculate the propensity score for 2130 of the 2163 very preterm infants who were alive at hospital discharge and whose status regarding breast feeding status at time of discharge was known. The propensity scores ranged from 0.0005 to 0.722. The Hosmer-Lemeshow test was 7.2, p=0.51. The ROC curve area was 0.72 ± 0.01. In LIFT cohort, the propensity score was calculated for all preterm infants. For each variable, if necessary, a subgroup with unknown data was constituted. The propensity scores ranged from 0.007 to 0.747. The Hosmer-Lemeshow test was 6.2, p=0.63. The ROC curve area was 0.71 ± 0.01. Variables significantly associated with breastfeeding at discharge were very similar in both cohorts (table 2).

**Exposure to Breastfeeding at Time of Discharge and neurodevelopmental Outcome**

In both cohorts, breastfeeding was associated with a significant reduction of risk for a suboptimal neurodevelopmental assessment at 2 years of corrected age (LIFT cohort) and at 5 years, (Table 3). Moreover, The K-ABC Mental Processing Composite score increased as a function of the corrected age at which infants were weaned off breastfeeding (Fig 2). In each cohort, breastfeeding was consistently associated with a reduction in the risk for suboptimal neurodevelopmental assessment before and after adjustment for gestational age, birth weight Z-score and sex, and propensity score (Table 3). **We observed the same results in propensity - score matching analysis (Supplemental table).**

**Weight gain during neonatal hospitalization and exposure to breastfeeding.**

Breastfeeding was associated with an increased risk of losing one weight Z-Score during hospitalization, before adjustment in LIFT cohort, and after adjustment in both cohorts (Table 4). The loss in weight Z-Score during NICU hospitalization was similar in both cohorts lin breastfed infants but not in non breastfed infants: the loss was less pronounced in the most recent cohort.
We observed a greater loss in weight Z-Score in breastfed group in propensity score matching analysis, and this for each cohort (Supplemental table).

Weight gain during neonatal hospitalization, exposure to breastfeeding, and neurodevelopmental outcome.

Restricted intrauterine growth (i.e., lower birth weight Z Score) was associated before and after adjustment for gestational age and sex with a suboptimal neurodevelopmental assessment in both cohorts. Postnatal weight gain during neonatal hospitalization was significantly associated with a suboptimal neurodevelopmental assessment only in LIFT cohort but not in EPIPAGE cohort (Table 5), with and without adjustment for gestational age, birth weight Z-score, sex, breastfeeding and propensity score.

Growth after neonatal hospitalization.

In contrast with data on initial growth, from 2 years of corrected age, weight, height and head circumference were significantly higher in preterm infants who had been breastfed at the time of discharge in both LIFT and Epipage cohorts (Fig 2). After adjustment for weight Z Score at birth, sex and propensity score, breastfeeding at discharge was significantly associated to an increased chance of having a head circumference Z-score higher than 0.5 at five years in EPIPAGE cohort, (n=1412, aOR=1.47, [1.10-1.95]) and at two years of corrected age in LIFT cohort, (n=1276, aOR=1.43, [1.02-2.02]).

DISCUSSION

In two independent cohorts of preterm infants, breastfeeding at discharge was associated with a reduction in the risk for a suboptimal neurodevelopmental assessment at two (LIFT cohort) or five years of age (EPIPAGE cohort) despite a higher risk for suboptimal weight gain (loss of one weight Z-Score) during neonatal hospitalization in these breastfed infants. The observed improved neurodevelopment in spite of suboptimal initial weight gain could be termed the ‘apparent breastfeeding paradox’ in very preterm infants.

Such an observation is indeed a paradox as several earlier studies have documented an association between suboptimal early postnatal nutrition with insufficient weight gain during hospital stay and later cognitive dysfunction. In extremely preterm infants, faster weight gain in NICU was associated with improved outcome in terms of neurodevelopment and growth at 18 to
22 months’ corrected age. (3) Nevertheless, this paradox is probably only an apparent paradox because weight gain during hospitalization is a poor predictor of the quality of growth, as it does not provide any insight into the changes in body composition. In infants with a very low birth weight, enhanced postnatal growth is also associated with a better later neurodevelopmental outcome (18), especially regarding postnatal growth in head circumference, an index of brain growth (19,20). Yet the “neuroprotective” effects of improved growth may only be mild and mainly concern growth over the first few weeks of hospital stay (21). In the two cohorts used in the current analysis, the role of postnatal weight gain was slightly different. In the EPIPAGE cohort, we did not observe any association between postnatal weight gain during hospitalization and sub-optimal neurodevelopmental score at five years of age (Table 5). In the LIFT cohort, after adjustment for propensity score, a significant association was found between initial weight gain rate and neurodevelopment. In contrast, in both cohorts a negative association was obvious between birthweight Z-score and suboptimal neuro-development (Table 5). Such difference between the 2 cohorts could be in relation with the significantly better initial neonatal weight gain rate in the LIFT cohort, compared with the EPIPAGE cohort, as a matter of fact, the loss in weight Z-score was less in the most recent cohort, presumably due to improvement in care routines between the two periods (1997 vs. 2003) in the NICUs in France (22). Thanks to the improvement in nutritional management in the second period, the role of postnatal nutrition, adjusted for breastfeeding, may have become detectable in the more recent LIFT cohort.

Beneficial effects of breastfeeding on cognitive skills and behavioral scores have been demonstrated previously in term (23,24), preterm (25), and extremely preterm infants (5). Multiple biases may, however, interfere, particularly maternal socioeconomic and educational status: in the two cohorts, upper social status was obviously associated with a higher chance for being breastfed at discharge, and for an optimal neurodevelopmental score. In term infants, after control for biases (26)—particularly using the method of sibling comparison which automatically controls for any confounding factors that are the same for each of the siblings in a pair (27)—the role of breastfeeding has been found to be either not significant or modest. In preterm infants, observational studies have shown that preterm infants whose mothers chose to breastfeed reached a higher intelligence quotient at 8 years of age. Interestingly, infants whose mothers were willing to breastfeed but failed to do so had the same IQ as those whose mothers elected not to breastfeed (25). In preterm infants, observational studies found feeding with human milk to be associated with a better outcome (5). Eight randomized studies were included in a Cochrane review (28). Only 2 old randomized studies using unfortified donor human milk reported a comparison of developmental outcome between unfortified human milk and formula feeding. Follow-up of the infants who participated in these two trials did not find a significant
effect on long-term growth parameters or neurodevelopmental outcomes. Most of other
parameters reported by the randomized studies comparing formula to human milk were in favor
of breastmilk, except for weight gain during hospitalization. Moreover, among children who had
been enrolled in one of such studies Lucas et al observed 15 years later, that breastmilk
consumption was associated with a lower blood pressure in children born prematurely,
suggesting a long-term beneficial effects of breastmilk in preterm infants (29). Taken together,
data from the literature, and from the current study are consistent with a benefit of breastmilk
consumption on developmental outcome.

At the time of discharge, very preterm infants have accumulated deficits in energy, protein and
minerals, and, due to their early discharge, still have higher nutrient requirements than healthy
appropriate-for-gestational age term infants. Despite the vast body of published
recommendations on the nutrition of preterm infants, there is little data on optimal nutritional
management after discharge of breastfed preterm infants (30). No randomized studies can be
ethically performed to determine whether feeding preterm infants following hospital discharge
with nutrient-enriched formula milk versus human breast milk affects growth and development.
The conclusion of a Cochrane review (2) is that “mothers who wish to breast feed, and their
health care advisors, would require very clear evidence that feeding with a nutrient-enriched
formula milk had major advantages for their infants before electing not to feed (or to reduce
feeding) with maternal breast milk, and that evidence from trials that compared feeding preterm
infants following hospital discharge with nutrient-enriched versus standard formula milk
demonstrating an effect on growth or development “. The need to contribute an answer to the
nagging, disturbing question, ‘Does exclusive breastfeeding at the time of discharge influence
outcome?’ was the very incentive to perform the current study. Our analysis of two large cohorts
of preterm infant with relatively long term follow-up, used the propensity score method as a
means to control as much as possible for potential confounders. From such analysis we
obtained clear-cut evidence that despite an increased risk for suboptimal early weight gain
(increased risk for the loss of one weight Z-Score) during neonatal hospitalization, breastfeeding
at discharge is associated with a better outcome after adjustment for potential confounders by
using propensity score.

The main weakness of our study is the observational design. Despite the use of propensity
score we cannot ensure all potential confounders were eliminated. For instance, the very ability
of an infant to suckle may be associated with a less sick infant, and the variables used in
adjustment may not be entirely independent either of each other or of the outcome. Moreover,
how much fortifier was given could not, unfortunately, be retrieved from either of the two
databases, and thus some important variable such as caloric intake during neonatal intensive care unit stay could not be included in the analysis. Similarly, we did not address the mechanisms. For instance, the effect of breastfeeding for term infants has been attributed to bias (31), including the complex relationship between weight gain and timing of weaning. Such bias is unlikely to be relevant for our population since the preterm infants with the slower weight gain rate were those who were not weaned at discharge. The slower weight gain during neonatal intensive care unit stay therefore likely reflects a biological effect rather than bias.

Several potential mechanisms can be proposed to explain the better outcome in breastfed infants: mother-child interaction (better bonding, better care given by parents), the effect of specific nutrients contained in breastmilk such as polyunsaturated fatty acids, prebiotic oligosaccharides, etc. Regardless of mechanisms we did observe the same fact in two distinct cohorts, with the same magnitude. By adjustment for propensity score, sex, and birth weight Z-score, we were indeed able to observe the apparent paradox of a better neurodevelopmental outcome, despite a lower early weight gain. These adjustments are necessary because a complex relationship exists between breastfeeding, birth weight, gestational age, birth weight Z-score, and postnatal weight gain during hospitalization.

In conclusion, the neurodevelopment of premature infants is likely to benefit from feeding supplemented mother milk during hospital stay and unsupplemented mother’s milk after discharge, and these data from two cohorts are indeed reassuring, because in spite of a lesser weight gain during hospitalization, we observed a better neurodevelopmental outcome in the breastfed groups. The present report suggests breastfeeding should be recommended at the time of discharge. As the rate of exclusive breastfeeding at time of discharge is low—less than 30% in Europe (32)—strategies to facilitate breastfeeding at discharge must be developed (33). Moreover, when adjusted on breastfeeding, postnatal weight gain has a positive effect on neurodevelopmental outcome as observed in LIFT cohort; so the question about the putative benefit of human milk supplementation after discharge remains open. Supplementation must be continued as long as possible, according to the state of knowledge and this suggests also that more research is warranted about human milk composition and the potential benefit of human milk supplementation at time of discharge is warranted in the future.
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Author Contributions: Conceived and designed the experiments: JCR, DD, MK PYA. Analyzed the data: JCR, DD, MK, PYA, CYB. Wrote the paper: JCR, DD, PYA. Data acquisition: JCR, CF, JCP, CS, DM, BB, US. Revision and critical review of the manuscript: JCR, DD, JCP, AL, OC, DM, US. The Corresponding Author, JC Rozé has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive license on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in BMJ editions and any other BMJ products and sublicenses to exploit all subsidiary rights.
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Figure 1. Flow charts.
Figure 2. The K-ABC Mental Processing Composite score (mean, SD) at five years as a function of breastfeeding status at time of discharge and corrected age (\(^*\)) at which infants were weaned off breastfeeding, in EPIPAGE cohort.

\(^\ddagger\) p adjusted for propensity score.
Figure 3. Weight, head circumference and height expressed as Z-score at discharge, 6 or 9 months and 2 years of corrected age (EPIPAGE and LIFT cohorts), and at 5 years (EPIPAGE cohort) according to breastfeeding at discharge. Weight measurements was known for 1460, 1430, 973, 873, 1447 infants at birth, discharge, 6 months, 2 and 5 years respectively in EPIPAGE, and for 1463, 1463, 1341 and 1297 infants at birth, discharge, 9 months and 2 years respectively in LIFT cohort.
Table 1. Demographic and Clinical Characteristics of Very Low Gestational Age Infants Breastfed or Not at Time of Discharge.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EPIPAGE COHORT</th>
<th></th>
<th>LIFT COHORT</th>
<th></th>
<th>p value</th>
<th></th>
<th>LIFT COHORT</th>
<th></th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Breastfed</td>
<td>Not Breastfed</td>
<td>P value</td>
<td>Breastfed</td>
<td>Not Breastfed</td>
<td>P value</td>
<td>Breastfed</td>
<td>Not Breastfed</td>
<td>P value</td>
</tr>
<tr>
<td>Characteristics of newborns</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age, w</td>
<td>30.4 (1.8)</td>
<td>29.8 (2.1)</td>
<td>0.001</td>
<td>30.3 (1.8)</td>
<td>29.8 (2.1)</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>1460 (400)</td>
<td>1360 (390)</td>
<td>0.001</td>
<td>1430 (280)</td>
<td>1330 (400)</td>
<td>0.001</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Birth weight, Z-Score</td>
<td>-0.23 (1.15)</td>
<td>-0.28 (1.05)</td>
<td>0.26</td>
<td>-0.26 (0.81)</td>
<td>-0.34 (0.81)</td>
<td>0.120</td>
<td></td>
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</tr>
<tr>
<td>Male gender,</td>
<td>212 (51.8)</td>
<td>952 (54.3)</td>
<td>0.36</td>
<td>155 (55.8)</td>
<td>788 (54.2)</td>
<td>0.62</td>
<td></td>
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<tr>
<td>Characteristics of mothers</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Age less than 25 years</td>
<td>49 (12.0)</td>
<td>372 (21.2)</td>
<td>0.001</td>
<td>-</td>
<td>-</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Two or more children at home</td>
<td>57 (13.9)</td>
<td>349 (19.9)</td>
<td>0.005</td>
<td>121 (43.5)</td>
<td>675 (46.3)</td>
<td>0.013</td>
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<tr>
<td>Single</td>
<td>18 (4.4)</td>
<td>157 (9.0)</td>
<td>0.002</td>
<td>20 (7.2)</td>
<td>150 (10.3)</td>
<td>0.012</td>
<td></td>
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</tr>
<tr>
<td>Upper level education</td>
<td>178 (43.5)</td>
<td>430 (24.5)</td>
<td>0.001</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td>No professional activity</td>
<td>38 (9.3)</td>
<td>338 (19.3)</td>
<td>0.001</td>
<td>52 (18.7)</td>
<td>451 (31.0)</td>
<td>0.001</td>
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<tr>
<td>Health insurance for low financial income</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>12 (4.3)</td>
<td>146 (10.0)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper socio-demographic level</td>
<td>211 (51.6)</td>
<td>527 (30.0)</td>
<td>0.001</td>
<td>135 (48.6)</td>
<td>385 (26.5)</td>
<td>0.001</td>
<td></td>
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<tr>
<td>Characteristics of pregnancy</td>
<td></td>
<td></td>
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<tr>
<td>Hypertension during pregnancy</td>
<td>99 (24.2)</td>
<td>381 (21.7)</td>
<td>0.276</td>
<td>31 (11.2)</td>
<td>253 (17.4)</td>
<td>0.010</td>
<td></td>
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<tr>
<td>Multiple pregnancy</td>
<td>101 (24.7)</td>
<td>572 (32.6)</td>
<td>0.002</td>
<td>61 (21.9)</td>
<td>415 (28.5)</td>
<td>0.024</td>
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<tr>
<td>Neonatal Hospitalization</td>
<td></td>
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<tr>
<td>Duration of mechanical ventilation, days</td>
<td>3.1 (7.5)</td>
<td>5.6 (11.5)</td>
<td>0.001</td>
<td>4.0 (10.4)</td>
<td>6.8 (11.2)</td>
<td>0.002</td>
<td></td>
<td></td>
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<tr>
<td>Length of hospital stay, days</td>
<td>52.5 (24.1)</td>
<td>62.2 (32.6)</td>
<td>0.001</td>
<td>54.2 (29.2)</td>
<td>59.5 (32.7)</td>
<td>0.013</td>
<td></td>
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</tr>
<tr>
<td>Change in weight Z-score during neonatal</td>
<td>-1.00 (0.63)</td>
<td>-0.95 (0.63)</td>
<td>0.170</td>
<td>-1.02 (0.60)</td>
<td>-0.80 (0.70)</td>
<td>0.001</td>
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<tr>
<td>hospitalization,</td>
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<tr>
<td>Follow-up &amp; Outcome</td>
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<tr>
<td>Lost to follow-up</td>
<td>49 (12.0)</td>
<td>361 (20.6)</td>
<td>0.001</td>
<td>22 (7.9)</td>
<td>124 (8.5)</td>
<td>0.738</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Follow-up</td>
<td>360 (88.0)</td>
<td>1393 (79.4)</td>
<td>0.001</td>
<td>256 (92.1)</td>
<td>1331 (91.5)</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incomplete neuro-developmental evaluation</td>
<td>44 (12.2)</td>
<td>247 (17.7)</td>
<td>0.001</td>
<td>14 (5.5)</td>
<td>110 (8.3)</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal neuro-development</td>
<td>252 (70.0)</td>
<td>725 (52.0)</td>
<td>0.001</td>
<td>183 (71.5)</td>
<td>761 (57.2)</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non Optimal neuro-development</td>
<td>64 (17.8)</td>
<td>421 (30.2)</td>
<td>0.001</td>
<td>59 (23.0)</td>
<td>460 (34.5)</td>
<td>0.001</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Data are means (SD) or n (%). Non optimal neuro-development was defined as a KABC mental composite processing less than 85 at five years in EPIPAGE cohort and an ASQ score less than 220 at two years of corrected age in LIFT cohort.
Table 2. Significant Associations between the Variables Included in Calculation of Propensity Score and Breast feeding.

<table>
<thead>
<tr>
<th>Characteristics of the mothers</th>
<th>EPIPAGE COHORT</th>
<th>LIFT COHORT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=2130</td>
<td>n=1733</td>
</tr>
<tr>
<td></td>
<td>aOR 95%CI P</td>
<td>aOR 95%CI P</td>
</tr>
<tr>
<td>Age less than 25 years</td>
<td>0.61 (0.42 - 0.89) 0.010</td>
<td>-</td>
</tr>
<tr>
<td>Upper socio-demographic level</td>
<td>1.77 (1.34 - 2.34) 0.001</td>
<td>2.32 (1.70 - 3.17) 0.001</td>
</tr>
<tr>
<td>Higher education</td>
<td>1.55 (1.17 - 2.06) 0.002</td>
<td>-</td>
</tr>
<tr>
<td>No Professional activity</td>
<td>0.66 (0.44 - 1.00) 0.052</td>
<td>0.57 (0.35 - 0.75) 0.001</td>
</tr>
<tr>
<td>Mother of foreign origin</td>
<td>1.50 (1.05 - 2.14) 0.024</td>
<td>-</td>
</tr>
<tr>
<td>Characteristics of Pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>0.51 (0.39 - 0.67) 0.001</td>
<td>0.69 (0.48 - 0.99) 0.047</td>
</tr>
<tr>
<td>Characteristics of the newborns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of neonatal hospitalization (by week)</td>
<td>0.89 (0.84 - 0.94) 0.001</td>
<td>0.91 (0.85 - 0.97) 0.004</td>
</tr>
<tr>
<td>Gestational age (per week)</td>
<td>1.03 (0.92 - 1.16) 0.650</td>
<td>0.98 (0.86 - 1.10) 0.714</td>
</tr>
<tr>
<td>Birth weight (per Kg)</td>
<td>1.17 (0.66 - 2.08) 0.590</td>
<td>1.13 (0.70 - 1.83) 0.612</td>
</tr>
</tbody>
</table>

aOR, adjusted odd ratio
Table 3. Association between breast feeding at time of discharge and non optimal neuro-developmental performance.

<table>
<thead>
<tr>
<th>Adjustment</th>
<th>EPIPAGE Cohort n=1462</th>
<th></th>
<th></th>
<th>LIFT Cohort n=1463</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95%CI</td>
<td>p</td>
<td>OR</td>
<td>95%CI</td>
<td>P</td>
</tr>
<tr>
<td>No adjustment</td>
<td>0.44</td>
<td>(0.33 - 0.60)</td>
<td>0.001</td>
<td>0.53</td>
<td>(0.39-0.73)</td>
<td>0.001</td>
</tr>
<tr>
<td>Adjusted for gestational age, birth weight Z-score and sex.</td>
<td>0.46</td>
<td>(0.34 – 0.62)</td>
<td>0.001</td>
<td>0.57</td>
<td>(0.41-0.78)</td>
<td>0.001</td>
</tr>
<tr>
<td>Adjusted for gestational age, birth weight Z-score, sex and propensity score*</td>
<td>0.65</td>
<td>(0.47 – 0.89)</td>
<td>0.008</td>
<td>0.63</td>
<td>(0.45-0.87)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Non optimal neuro-development was defined as a KABC mental composite processing less than 85 at five years in EPIPAGE cohort and a ASQ score less than 220 at two years of corrected age in LIFT cohort.

*n=1443.
Table 4. Association between breast feeding at time of discharge and slow postnatal growth defined as a loss of one Z-score unit for weight during neonatal hospitalization.

<table>
<thead>
<tr>
<th>Adjustment</th>
<th>EPiPAGE Cohort</th>
<th>LIFT Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=1430</td>
<td>n=1463</td>
</tr>
<tr>
<td>No adjustment</td>
<td>1.12 (0.87 - 1.43)</td>
<td>1.92 (1.49-2.49)</td>
</tr>
<tr>
<td>Adjusted for gestational age, sex, birth weight Z-score</td>
<td>1.32 (0.98 – 1.76)</td>
<td>2.66 (1.99-3.52)</td>
</tr>
<tr>
<td>Adjusted for gestational age, sex, birth weight Z-score and propensity score*</td>
<td>1.55 (1.14 - 2.12)</td>
<td>2.51 (1.87-3.36)</td>
</tr>
</tbody>
</table>

* n =1417.
Table 5. Association between birth weight or postnatal growth and non optimal neuro-development.

<table>
<thead>
<tr>
<th></th>
<th>EPIPAGE Cohort</th>
<th>LIFT Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Birth weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(per one Z-score unit below the mean)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No adjustment</td>
<td>1.19</td>
<td>(1.04-1.35)</td>
</tr>
<tr>
<td>Adjusted for gestational age, sex</td>
<td>1.23</td>
<td>(1.07-1.40)</td>
</tr>
<tr>
<td>Postnatal Growth for weight</td>
<td>(per one Z-score unit lost between birth and discharge)</td>
<td>n=1430</td>
</tr>
<tr>
<td>No adjustment</td>
<td>0.93</td>
<td>(0.78 - 1.11)</td>
</tr>
<tr>
<td>Adjusted for gestational age, sex, birth weight Z-score</td>
<td>0.97</td>
<td>(0.79 - 1.19)</td>
</tr>
<tr>
<td>Adjusted for gestational age, sex, birth weight Z-score, breastfeeding and propensity score *</td>
<td>1.06</td>
<td>(0.85 - 1.31)</td>
</tr>
</tbody>
</table>

Non optimal neuro-development was defined as a KABC mental composite processing less than 85 at five years in EPIPAGE cohort and a ASQ score less than 220 at two years of corrected age in LIFT cohort.

*n =1417
### Supplemental table. Demographic, Clinical Characteristics and Outcome of Very Low Gestational Age Infants Breastfed or Not at Time of Discharge in Matched pairs of each Cohort.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EPIPAGE COHORT</th>
<th>LIFT COHORT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Breastfed n=386</td>
<td>Not Breastfed n=386</td>
</tr>
<tr>
<td>Propensity score</td>
<td>0.251 (0.117)</td>
<td>0.251 (0.117)</td>
</tr>
<tr>
<td>Characteristics of newborns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age, w</td>
<td>30.3 (1.8)</td>
<td>30.2 (2.0)</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>1440 (400)</td>
<td>1450 (400)</td>
</tr>
<tr>
<td>Birth weight, Z-Score</td>
<td>-0.25 (0.87)</td>
<td>-0.17 (0.82)</td>
</tr>
<tr>
<td>Male gender, Z-Score</td>
<td>200 (51.8)</td>
<td>214 (55.4)</td>
</tr>
<tr>
<td>Characteristics of mothers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age less than 25 years</td>
<td>46 (11.9)</td>
<td>46 (11.9)</td>
</tr>
<tr>
<td>Two or more children at home</td>
<td>54 (14.0)</td>
<td>60 (15.5)</td>
</tr>
<tr>
<td>Single</td>
<td>18 (4.7)</td>
<td>18 (4.7)</td>
</tr>
<tr>
<td>Upper level education</td>
<td>164 (42.5)</td>
<td>153 (39.6)</td>
</tr>
<tr>
<td>No professional activity</td>
<td>36 (9.3)</td>
<td>31 (8.0)</td>
</tr>
<tr>
<td>Health insurance for low financial income</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Upper socio-demographic level</td>
<td>197 (51.0)</td>
<td>152 (39.4)</td>
</tr>
<tr>
<td>Characteristics of pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension during pregnancy</td>
<td>95 (24.6)</td>
<td>84 (21.8)</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>99 (25.6)</td>
<td>102 (26.4)</td>
</tr>
<tr>
<td>Neonatal Hospitalization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of mechanical ventilation, days</td>
<td>3.3 (7.7)</td>
<td>3.7 (8.1)</td>
</tr>
<tr>
<td>Length of hospital stay, days</td>
<td>53.4 (24.3)</td>
<td>54.2 (24.9)</td>
</tr>
<tr>
<td>Change in weight Z-score during neonatal hospitalization</td>
<td>-1.01 (0.64)</td>
<td>-0.91 (0.61)</td>
</tr>
<tr>
<td>Follow-up &amp; Outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>44 (11.6)</td>
<td>65 (16.8)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>342 (88.6)</td>
<td>321 (83.2)</td>
</tr>
<tr>
<td>Incomplete neuro-developmental evaluation</td>
<td>41 (12.2)</td>
<td>52 (17.7)</td>
</tr>
<tr>
<td>Normal neuro-development</td>
<td>239 (69.9)</td>
<td>192 (59.8)</td>
</tr>
<tr>
<td>Non Optimal neuro-development</td>
<td>62 (18.7)</td>
<td>77 (24.0)</td>
</tr>
</tbody>
</table>

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Flow charts

90x65mm (600 x 600 DPI)
The K-ABC Mental Processing Composite score (mean,SD) at five years as a function of breastfeeding status at time of discharge and corrected age (*) at which infants were weaned off breastfeeding, in EPIPAGE cohort.

‡ p adjusted for propensity score.

122x91mm (600 x 600 DPI)
Weight, head circumference and height expressed as Z-score at discharge, 6 or 9 months and 2 years of corrected age (EPIPAGE and LIFT cohorts), and at 5 years (EPIPAGE cohort) according to breastfeeding at discharge. Weight measurements was known for 1460, 1430, 973, 873,1447 infants at birth, discharge, 6 months, 2 and 5 years respectively in EPIPAGE, and for 1463,1463,1341 and 1297 infants at birth, discharge, 9 months and 2 years respectively in LIFT cohort.

65x75mm (600 x 600 DPI)
<table>
<thead>
<tr>
<th>Item No</th>
<th>Recommendation</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Title and abstract</strong></td>
<td>1</td>
<td>(a) Indicate the study’s design with a commonly used term in the title or the abstract</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Provide in the abstract an informative and balanced summary of what was done and what was found</td>
</tr>
<tr>
<td><strong>Introduction</strong></td>
<td>2</td>
<td>Explain the scientific background and rationale for the investigation being reported</td>
</tr>
<tr>
<td><strong>Objectives</strong></td>
<td>3</td>
<td>State specific objectives, including any prespecified hypotheses</td>
</tr>
<tr>
<td><strong>Methods</strong></td>
<td>4</td>
<td>Present key elements of study design early in the paper</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) For matched studies, give matching criteria and number of exposed and unexposed</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable</td>
</tr>
<tr>
<td></td>
<td>8*</td>
<td>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</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Describe any efforts to address potential sources of bias</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Explain how the study size was arrived at</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>(a) Describe all statistical methods, including those used to control for confounding</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Describe any methods used to examine subgroups and interactions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Explain how missing data were addressed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) If applicable, explain how loss to follow-up was addressed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e) Describe any sensitivity analyses</td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td>13*</td>
<td>(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</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Give reasons for non-participation at each stage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Consider use of a flow diagram</td>
</tr>
<tr>
<td></td>
<td>14*</td>
<td>(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Indicate number of participants with missing data for each variable of interest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Summarise follow-up time (eg, average and total amount)</td>
</tr>
<tr>
<td></td>
<td>15*</td>
<td>Report numbers of outcome events or summary measures over time</td>
</tr>
<tr>
<td><strong>Main results</strong></td>
<td>16</td>
<td>(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</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Report category boundaries when continuous variables were categorized</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period</td>
</tr>
<tr>
<td><strong>Other analyses</strong></td>
<td>17</td>
<td>Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses</td>
</tr>
<tr>
<td>Discussion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Key results</td>
<td>Summarise key results with reference to study objectives</td>
<td></td>
</tr>
<tr>
<td>Limitations</td>
<td>Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias</td>
<td></td>
</tr>
<tr>
<td>Interpretation</td>
<td>Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence</td>
<td></td>
</tr>
<tr>
<td>Generalisability</td>
<td>Discuss the generalisability (external validity) of the study results</td>
<td></td>
</tr>
<tr>
<td>Other information</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Funding</td>
<td>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</td>
<td></td>
</tr>
</tbody>
</table>