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Breastfeeding duration, milk fat composition and developmental indices at 1 year of life among breastfed infants

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Summary The associations of breastfeeding duration and milk fat composition with the developmental outcome at 1 year of age were measured within 44 infants exclusively breastfed for 3 months, out of 95 recruited at birth. Pooled breast milk (hindmilk) of the mothers was analysed at colostrum, 1, 3, 6, 9, and 12 months for total fat and fatty acid content. Infants were examined at 12 months by means of the Bayley test. There was a progressive reduction of the number of breastfed babies after the introduction of solids to 29 (6 months), 17 (9 months) and 10 (12 months). After adjusting for major confounders, infants breastfed for 6 months or longer showed a trend to have an advantage at the Bayley psychomotor developmental index compared to those breastfed >3 and <6 months (95% CI for difference: $-0.6, 13.8$; $P=0.07$) while the Bayley mental developmental index (MDI) was just 2.1 points higher. Among the milk fat components considered for each time-point, the total fat content at 6 months showed the strongest association with the MDI at 12 months (r=0.59, P=0.001). Prolonging breastfeeding during the weaning process may result in a better developmental performance at 12 months, possibly due to the supply of fats affecting brain composition. © 2001 Harcourt Publishers Ltd

INTRODUCTION

Autopsy studies have shown that dietary fats may affect brain composition in early life.[1,2] Indeed, human milk supplies LCPUFA such as docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) that are absent in most standard formulas for term infants and is associated with a higher LCPUFA content in brain. The unique n-6 and n-3 long-chain polyunsaturated fatty acid (LCPUFA) composition of human milk[3] has been proposed to result in higher developmental scores in breastfed infants[4] and in LCPUFA-enriched formula-fed infants compared to standard formula-fed ones at short- and medium-term assessments as shown in randomized studies.[5,6] The amounts of LCPUFA that are supplied to suckling infants are strictly dependent on the duration of breastfeeding and their levels in human milk. Even when solids are introduced during the weaning period, the molecular forms in which LCPUFA are complexed in human milk[3] could make them more easily available for biological processes. At present it is not known whether the duration of breastfeeding and/or the different fat content and fatty acid (FA) composition of human milk may affect developmental indices within breastfed infants.

In this study developmental indices at 1 year of age were evaluated in relation to the duration of breastfeeding and the milk fat composition within infants exclusively breastfed for at least 3 months.

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SUBJECTS AND METHODS

The primary aim was the evaluation of the human milk FA composition and its associations with the infants' developmental outcome from at least 10 mothers throughout 12-month nursing. A sufficient number of mothers were recruited at delivery on the basis of the epidemiological data on the duration of the breastfeeding practice in our area (Northwestern Italy) indicating that around 10% mothers are still breastfeeding 12 months after delivery.[7] On this basis, we expected 50% of mothers exclusively breastfeeding at 3 months and 20% breastfeeding at 6 months. To have a r value of 0.60 (α=0.05, power=90%) at least 21 subjects are necessary, while 10 subjects would be able to detect a r value >0.70 with the same α and power=80%. Inclusion criterion for the infants' developmental evaluation was to be exclusively breastfed for 3 months.

Mothers who gave birth to healthy, full-term infants (37–42 weeks gestation) in our hospital were eligible for entry into the study. Women suffering from any metabolic disorder (hyperlipidaemia, insulin-dependent diabetes) and/or taking corticosteroids during pregnancy were excluded. Mothers gave informed consent and the design was approved by the Institutional Ethical Committee.

Mothers' characteristics (number of deliveries, age, usual smoking habits) were recorded at recruitment as potential confounders. Familial social status (based on father's occupation) and maternal education were coded according to the Italian Census and included into two categories (lower vs middle and upper class, elementary vs secondary and university education). Infants were seen at 1 month and 3, 6, 9 and 12 months of age. At each visit the infants' type of feeding was assessed by interviewing the mothers. Weaning foods were introduced from the 5th month onwards in all the subjects with a dietary schedule reflecting the weaning habits in our area.[8]

At 12 months ($\pm$10 days), post-natal age infants entering the inclusion criterion were examined by a monitor unaware of the infants' type of feeding through the Bayley Scales of Infant Development. Infants were given a mental developmental index (MDI) and a psychomotor developmental index (PDI).[9]

All participant mothers were encouraged to breastfeed for as long as possible and were instructed to express breast milk at the end of each feed (hindmilk) over a 24-h period into sterile vials the day before the infants' control. Hindmilk collection is less troublesome to mothers since it does not interfere with infant suction. To validate our findings, seven mothers were requested to collect fore-, midstream- and hindmilk during each suckling episode throughout 24 h at colostrum, 1 month- and 3 month-lactation. The mean fat content of hindmilk for each mother was constantly higher than that of midstream milk (ranging from 25% to 37%) so we could conclude that hindmilk was representative of the fat contribution of each mother's milk. By attributing to the mid flow milk the 100% of fats we have found that foremilk and hindmilk contributed on average 70% and 132% respectively of fat concentrations of the midstream milk.

Breast milk samples were immediately frozen when collected and delivered to us for analysis when the participants brought their infants the day after milk collection. Aliquots from each meal of the day of collection were then pooled and analysed for total fatty acids. Milk lipid content and composition were determined at each time-point.

Milk total lipids were determined gravimetrically after extraction with chloroform-methanol (2:1) containing BHT (butyl-hydroxytoluene) as antioxidant, according to the method of Folch.[10] Fatty acids have been analysed as methyl esters by gas-chromatography using an SP-2560 Supelco capillary column (100 m length, 0.25 mm ID, 0.2 μm film thickness) with programmed temperature (from 150°C to 220°C). An internal standard (eptadecanoic acid, 17 : 0) has been added for quantitative analysis. For the purposes of the study both the absolute content (mg/dL) and the relative concentration (as total fatty acid %) were calculated for each fatty acid. Total n-6 LCPUFA included 20:3, 20:4, 22:4 and 22:5. Total n-3 LCPUFA included 20:5, 22:5 and 22:6.

For the statistical analyses Student's t test, ANOVA, Pearsons's or Spearman's (if $n \leq 20$) r and multiple stepwise regression analysis were used. Factors considered in bivariate analyses as independent variables were DHA, AA, total n-6 and total n-3 LCPUFA and total milk lipid content, with MDI and PDI as dependent variables. When two or more factors were related to either outcome variable they entered a multiple stepwise regression model. Data are expressed as means, SD and ranges (minimum–maximum) in Tables and described with 95% CI through the text.

RESULTS

Ninety-five mothers were recruited. Among them, 10 mothers completed the follow-up of 12-month nursing. Forty-four infants (24 males and 20 females) were exclusively breastfed for 3 months.

There was a progressive reduction of the number of breastfed babies to 29 (6 months), 17 (9 months) and 10 (12 months). These numbers are consistent with our findings on breastfeeding rates through 1 year in North-Western regions and Italy.[7,11]

The mean MDI and PDI values at 12 months are shown in Table 1. In Table 2 we report the mean unadjusted MDI and PDI values according to breastfeeding duration. After adjusting for potential confounders (whose distribution is shown in Table 3) breastfeeding for 6 months or longer still gives an advantage of 6.6 points at the Bayley PDI (95% CI for means=90.0–98.5 vs 81.6–93.5, 95% CI for

Table 1 Developmental indices at 12 months in the study population ($n = 44$)

	Mean	SD	Range
MDI	92.0	11.3	73–117
PDI	93.3	8.1	77–117

Table 2 Mean unadjusted PDI and MDI values at 12 months of age (mean, SD) according to breastfeeding duration

Duration	*n*	MDI	PDI
≥6 months	29	94.3, 10.9*	94.0, 7.4
3–6 months	15	87.4, 11.0*	92.1, 9.5
≥9 months	17	95.0, 11.9	92.8, 6.1
3–9 months	27	90.0,10.7	93.7, 9.2
≥12 months	10	92.5, 11.3	92.2, 5.7
3–12 months	34	91.8, 11.5	93.7, 8.7

* *t*-test, $P = 0.05$; 95% CI for difference = 0.0,14.0.

Table 3 Distribution of confounders among the 44 mothers

Age (<30 years, >30 years)	22, 22
Education (low, high)	16, 28
Social class (low, high)	13, 31
Parity (0, >1)	31, 13
Usual smokers (yes, not)	13, 31

Table 4

a Average fat content (g/L) of human milk at the different time-points

Time	*n*	Mean	SD	range
1 day	44	16.6	9.2	4.8–50.3
1 month	44	45.5	20.8	13.3–103.0
3 months	44	42.8	18.8	13.2–93.0
6 months	29	43.1	29.2	10.2–118.0
9 months	17	39.6	19.4	16.7–87.4
12 months	10	46.4	31.3	7.2–95.7

b Within-group correlations of milk fat content between two consecutive time-points

Period	*n*	*r**	*P*
1d–1 month	44	−0.14	0.35
1–3 months	44	0.10	0.51
3–6 months	29	0.56	0.002
6–9 months	17	0.40	0.10
9–12 months	10	0.65	0.04

*Pearson's *r* if more than 20 subjects, Spearman's *r* when less than 20 subjects.

difference=−0.6–13.8; *P*=0.07) and 2.1 points at the Bayley MDI (95% CI for difference=−3.2, 7.3: non-significant) compared to the 15 subjects breastfed less than 6 months.

The average fat content of human milk at the different time-points and the within-group correlations of milk fat content between two consecutive time-points are reported in Tables 4a and 4b. Associations between the milk fat content and LCPUFA composition at each time of milk collection and developmental indices have been looked for. At bivariate analysis positive associations with MDI at 12 months were found for fat content (r=0.59, P=0.001) (Fig. 1), n-6 LCPUFA (r=0.46, P=0.01) and n-3 LCPUFA (r=0.40, P=0.02) at 6 months and with the fat content at 12 months (r=0.58, P=0.07) (Fig. 2). After stepwise multiple regression analysis the human milk fat content was the only component of human milk at 6 months found associated to the 12-month MDI (B = 0.15, β = 0.59, P = 0.0007).

No associations between the milk components and the PDI scores were found at any time-point. Neither the DHA nor the AA content (as absolute weight or fatty acid%) at any time-point showed significant associations with both the 12-month MDI and PDI.

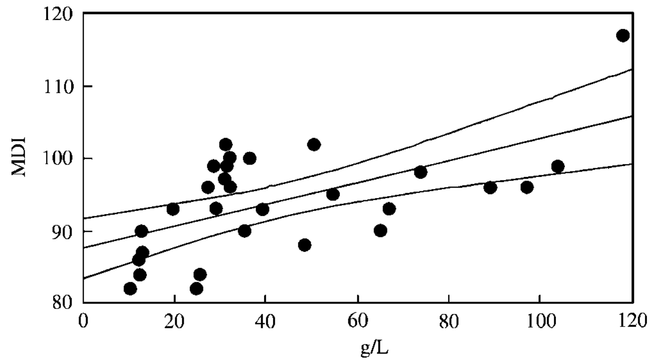

Fig. 1 Association between total fat content (g/L) in milk at 6 months with the 12-month MDI (mean and 95% CI outer lines, Rsq=0.354).

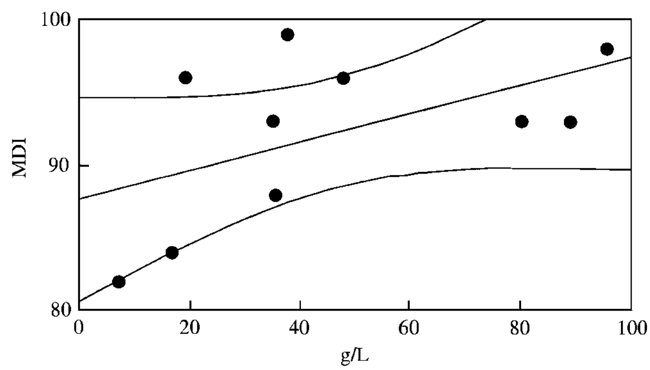

Fig. 2 Association between total fat content (g/L) at 12 months with the 12-month MDI (mean and 95% CI outer lines, Rsq=0.283).

DISCUSSION

Within an infant population exclusively breastfed for at least 3 months, we have found a difference in the Bayley

PDI at 12 months, according to breastfeeding duration longer than 6 months. The difference is mildly reduced after adjusting for confounders. Moreover, the lipid content of human milk at 6 months is associated with the MDI score at 12 months. Since mothers show a trend to maintain their milk fat levels between two-consecutive time-points (particularly during weaning) we speculate that infants that are breastfed for at least 6 months and whose mothers produce a high-fat milk may have an advantage at the short-term outcome. The wide inter-individual difference in the milk fat content that we have found (changing around ten times from the lowest to the highest value) is not unexpected, since it has been shown in other surveys on breastfeeding and milk composition.[12]

A large body of literature has investigated the effects of human milk on later development. It has been speculated that the positive effects of human milk on later infant development could be due to either nutritive characteristics or the close contact of infants to breastfeeding mothers.[13] Moreover, it is well known that several confounding factors are associated with the mothers' choice to breastfeed their infants.[14] A positive maternal attitude towards breastfeeding could also benefit the infants' mental development through improved bonding and nurturing behaviour, and cannot be a priori excluded. This association is supported by investigators who found a positive effect on the psychomotor and mental development of healthy term infants only in the case of prolonged breastfeeding.[15] We should also consider the possibility that mothers breastfeeding for a longer period have a better lifestyle and follow more closely dietary recommendations, possibly influencing their milk fat composition. Thus, the difference we have found in PDI scores, even if persisting after correction for some potential confounders, could be explained by factors other than the simple supply of maternal milk. The findings also support the hypothesis of a positive effect of human milk in terms of nutrient composition.

Several mechanisms have been proposed to explain the biofunctional role of LCPUFA in the early stages of development.[16] According to our present observations, the main lipid factor showing an influence on the short-term outcome is the absolute amount of fat in mothers' milk during weaning. In this period infants' dietary schedules in Italy provide the introduction of selected foods (vegetables, cereals, fruit, restricted types of cheese and lean meat), resulting in hypolipidic diets.[17] Thus, a greater energy intake from human milk fats during this crucial stage could result in a more rapid achievement of some developmental steps. Nevertheless, we cannot exclude a role for selected fatty acids supplied by human milk, since experiments have shown that the lipid composition of tissue membranes is influenced by the type of dietary fats during weaning.[18]

The opportunity to enrich the diet of breastfeeding mothers with LCPUFA in order to improve the developmental outcome has been explored in recent studies with weak functional results.[19,20] It can be speculated that the simple enrichment of human milk with DHA may not be effective unless total fat content of mothers' milk is also increased. Further studies on larger groups of breast fed infants are needed to confirm these preliminary observations.

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