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ORIGINAL COMMUNICATION

Breast milk and energy intake in exclusively, predominantly, and partially breast-fed infants

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Objective: To investigate the extent to which breast milk is replaced by intake of other liquids or foods, and to estimate energy intake of infants defined as exclusively (EBF), predominantly (PBF) and partially breast-fed (PartBF).
Design: Cross-sectional.
Setting: Community-based study in urban Pelotas, Southern Brazil.
Subjects: A total of 70 infants aged 4 months recruited at birth.
Main outcome measures: Breast milk intake measured using a 'dose-to-the-mother' deuterium-oxide turnover method; feeding pattern and macronutrient intake assessed using a frequency questionnaire.
Results: Adjusted mean breast milk intakes were not different between EBF and PBF (EBF, 806 g/day *vs* PBF, 778 g/day, $P=0.59$). The difference between EBF and PartBF was significant (PartBF, 603 g/day, $P=0.004$). Mean intakes of water from supplements were 10 g/day (EBF), 134 g/day (PBF) and 395 g/day (PartBF). Compared to EBF these differences were significant (EBF *vs* PBF, $P=0.005$; EBF *vs* PartBF, $P<0.001$).
The energy intake of infants receiving cow or formula milk (BF + CM/FM) in addition to breast milk tended to be 20% higher than the energy intake of EBF infants (EBF, 347 kJ/kg/day *vs* BF + CM/FM, 418 kJ/kg/day, $P=0.11$).
Conclusions: There was no evidence that breast milk was replaced by water, tea or juice in PBF compared to EBF infants. The energy intake in BF + CM/FM infants tended to be 20% above the latest recommendations (1996) for breast-fed and 9% above those for formula-fed infants. If high intakes are maintained, this may result in obesity later in life.
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Keywords: breast milk intake; deuterium-oxide turnover method; energy intake

Introduction

Exclusive breast-feeding (EBF), that is, intake of nothing but breast milk, is now recommended during the first 6 months of an infant's life (WHO, 2001a, b). However, in many societies infants are predominantly breast-fed (PBF), that is to say they receive water, tea and juices (Labbok & Krasovec, 1990) in addition to receiving milk from breast-feeding. There is evidence that the early introduction of complementary foods reduces levels of breast milk intake (Heinig *et al*, 1993), and others have found an association between the introduction of formula foods and early termination of breast-feeding (WHO, 1998a). It is also thought that even the introduction of non-nutritious liquids decreases breast milk production (Sachdev *et al*, 1991), but the precise extent to which breast milk intake is replaced by other liquids, milk or complementary foods remains a matter of some uncertainty.

In the past few years, there has been considerable renewed interest in the growth and energy requirements of breast-fed infants. Breast-fed infants tend to gain weight more rapidly in the first 2–3 months, but tend to weigh less than formula-fed infants between 6 and 12 months of age (WHO, 1994; Dewey *et al*, 1995). This needs to be taken into account when constructing growth curves for infants. To this end, WHO is currently undertaking the development of new growth

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curves (WHO, 1998b), which are intended to replace those developed by the National Center for Health Statistics (NCHS) (Hamill *et al*, 1977) and their recently revised version as developed by the Center for Disease Control (CDC) (Kuczmarski *et al*, 2002). The new WHO growth curves will be based on breast-fed infants from the high socioeconomic class with no constraints to growth, whereas the NCHS and CDC curves were derived from a mixture of breast- and bottle-fed infants from all social levels. Although growth curves might ideally be based on infants who are exclusively breast-fed for the first 4–6 months, in practice this would significantly complicate data collection in many countries including Brazil, where about 20% of the 3-month-old infants are PBF rather than EBF. Results for infants who are reported as EBF and PBF will therefore be pooled in the analysis of the WHO Multicenter Growth Reference Study (MGRS) on the basis that there is no difference in growth between these groups (Victora *et al*, 1998; De Onis *et al*, 2001). Partially breast-fed infants (PartBF), that is, infants receiving cow or formula milk and/or solids in addition to breast milk, did show increased weight gain during the first year of life compared to EBF or PBF infants (Victora *et al*, 1998). Infants partially breast-fed before 4 months of age will not be included in the database used for the construction of the new growth curves.

In line with these differences in growth is the finding that energy requirements of breast-fed infants are different from those who are bottle-fed (Butte *et al*, 1990; Butte, 1996), and there is substantial evidence that recommendations of energy requirements (WHO, 1985) overestimate the needs by 9–39% (Butte, 1996). Energy requirements have originally been based on measurements of energy intake (WHO, 1985). However, as it is difficult to obtain reliable food intake data, it was decided that energy requirements should be based on measurements of energy expenditure with an added component for growth of new tissue (IDECG, 1996). EBF infants may be a select group where reliable intake data can be obtained, and hence an accurate estimation of their energy requirements could be based on intake. We used the dose-to-the-mother deuterium-oxide turnover method to measure and compare intake of breast milk, and water from non-breast milk sources in EBF, PBF and PartBF infants aged 4 months. Our main objective was to investigate the extent to which breast milk is being replaced by the consumption of water, teas, juice, other milk or complementary foods. The age of 4 months was chosen as the latest possible age at which EBF would be common enough to achieve the sample size required. The study was designed to use the same inclusion criteria as the MGRS, so that results could be applied to the reference population included in the MGRS in Brazil. A second objective of the study was to estimate the energy intake of infants who were exclusively, predominantly or partially breast-fed.

The study was embedded in a lactation counselling intervention. Results of this work have been described elsewhere (Albernaz *et al*, 2003).

Methods

Outline of study milestones

The study was conducted in Pelotas, a city of about 330 000 habitants and 6000 births per year in the extreme south of Brazil (32°S and 52°W). Screening of subjects was carried out in the hospital at birth, from August 1999 to January 2000. The infants were followed up in their homes by two trained field-workers at 14, 30, 45, 60, 90 and 120 days of age, using the standard MGRS questionnaires (which contain information about socioeconomic family conditions, babies' and mothers' health as well as a 24 h recall) and measuring weight and length of mother and baby. At the time those infants eligible and breast-feeding were 105–120 days of age, breast milk intake was measured over a 2-week period using the deuterium-oxide turnover technique. A third field-worker was trained to: (1) administer the dose of deuterium-oxide to the mother on day 0, (2) collect saliva samples from the mother, (3) collect urine samples from the baby, (4) weigh the mother and the baby on days 0 and 14 of the study, and (5) apply a food frequency and amount questionnaire (FQ) on day 14 of the study covering days 0–14.

Subjects

The study used the same inclusion criteria as applied in the WHO Multicenter Growth Reference Study (WHO, 1998b) as previously carried out at the Pelotas research centre. Every day of the week, mother–infant pairs were recruited from three main hospitals. Eligibility criteria were (De Onis *et al*, 2001): (1) the mothers were living in the urban area of Pelotas, were non-smokers and were willing to breast-feed; (2) the babies were single births, gestational age was between 37 and 42 weeks, and the postnatal stay at the intensive care unit was <24 h; (3) family income was more than R$ 800 (reais). (At the time of the MGRS R$800 was equivalent to about USD 800; at the time of our study this was USD 500 because of currency devaluation.) Mothers who introduced formula or cow milk during the first 14 days after birth and those who started smoking during this period were excluded from participation.

Quality control of the screening was carried out in each hospital for one whole day every 3 weeks throughout the screening process.

The study was approved by the ethical committee of the Universidade Federal de Pelotas, and informed consent was given by the parents. At the end of the study, a letter mentioning the volume of breast milk intake of their baby during the period of observation was sent to the mothers for their information.

Classification criteria for infant feeding

Consumption of non-breast milk liquids and complementary foods was assessed using a food frequency and amount questionnaire (FQ). This questionnaire was applied during the last day of the deuterium study, and mothers were asked to recall the number of days water, tea, juice, fruits and solids had been given during the 14 days of the study. In addition, mothers estimated the volume of non-breast milk liquids, and reported the portion of a fruit and number of spoons of solid foods eaten during a meal. On the basis of this questionnaire, infants were classified by feeding pattern criteria during the 14 days of the study. These were (WHO, 1998b): (1) breast milk only (ie exclusively breast-fed) — EBF; (2) breast milk with teas, water, or juice given on at least 3 days a week (ie predominantly breast-fed) — PBF; (3) breast milk with formula or cow milk given every day — BM + FM/CM; (4) breast milk + complementary plementary foods given on at least 3 days a week — BM + CF; (5) breast milk plus formula or cow milk given every day and complementary foods given at least 3 days a week — BM + CF + FM/CM. Breast-fed children who received teas, water or other milk on an occasional basis were considered as EBF. For some statistical analyses, data were pooled from babies from the last three categories, and will be referred to as partially breast-fed (PartBF).

The records were coded by the field workers, and checked by a medical student for quality control.

Macronutrient intake

The volume of intake of water, tea, juice, formula and cow milk was estimated using the above-mentioned FQ. For this study, estimation of macronutrient intake was restricted to EBF, PBF and BF + CM/FM infants. Intake of energy (kJ/day), protein (g/day), fat (g/day), and carbohydrates (g/day) from tea, juice, cow milk and formula was estimated using a Brazilian food composition table (Instituto Brasileiro de Geografia e Estatistica, 1981). Macronutrient intake from breast milk was calculated using data on breast milk composition as given by WHO (WHO, 1998a). For energy this was 67 kcal/100 ml.

Measurements of breast milk intake

Breast milk intake was measured using the dose-to-the-mother deuterium-oxide turnover technique (Coward *et al*, 1982; Orr-Ewing *et al*, 1986; Butte *et al*, 1988). This technique also allows estimation of non-breast milk water intake, and the mother's body composition. A baseline sample of 2 ml of saliva from the mother and a urine sample from the child were collected on day 0, after which the mother received an oral dose of 10 g (0.5 mol) ^2H_2O, the quantity being determined to the nearest 0.01 g using an analytical Sartorius scale. A further three saliva samples from the mother (days 1,4,14) and another five urine samples from the baby (days 1,3,4,13,14) were then collected over a 14- day' period. Saliva collection was carried out after having been assured that the mother did not eat or drink in the previous 0.5 h. The time of collection was recorded. Small pieces of cotton wool were used to collect saliva samples (2 ml), after which saliva was released by compressing them in a syringe. For urine collection, urine samples (2 ml) were obtained as described elsewhere (Roberts & Lucas, 1985). Cotton wool balls were placed in clean diapers, which were then checked every 10 min. After urination the sample was collected from the cotton wool by compression in a syringe. Urine and saliva samples were stored on ice during transport on the days of field-work, and were stored in the field-workers' home freezer at the end of the day. When 1 week's samples had been collected, they were brought to the laboratory and stored at –20°C until the end of the study. At the end of the study all samples were sent by air to UK unfrozen.

^{2}H analysis

^{2}H enrichment in the saliva and urine samples was measured by isotope ratio mass spectrometry after equilibration with H_2 gas as described elsewhere (Hoffman *et al*, 2000). Each sample was measured in duplicate. The precision of the measurements was 0.26 ppm.

Calculations

Intake of breast milk and water from non-milk sources was calculated by fitting the isotopic (tracer) data to a model for water (tracee) turnover in the mothers and infants and the transfer of milk from mother to the baby (Coward *et al*, 1982; Orr-Ewing *et al*, 1986).

For the mother, data were fitted to

$$E_{m(t)} = E_{m(0)} e^{-K_{mm} t}$$

where $E_{m(t)}$ is isotopic enrichment above background at time t (ppm), $E_{m(0)}$ is the zero time isotope enrichment (ppm), t is time postdose (day) and K_{mm} is water turnover in the mother (1/day).

For the infant, data were fitted to

$$E_{b(t)} = E_{m(0)} \left(\frac{F_{bm}}{V_b}\right) \left(\frac{e^{-k_{mm} t} - e^{(F_{bb}/V_b)t}}{(F_{bb}/V_b) - K_{mm}}\right)$$

where $E_{b(t)}$ is isotopic enrichment above background at time t (ppm), F_{bm} is the transfer of water from the mother to the baby via breast milk (kg/day), V_b is the infant's total ^2H_2O distribution space (kg) and F_{bb} is total water loss in the baby (kg/day).

Curve fitting was performed by using the 'Solver' function in Excel® to minimise the sum of the squares of the differences between observed and fitted values for mother and baby data combined. Parameters fitted were $E_{m(0)}$, F_{bm}, K_{mm} and F_{bb}.. V_b was assumed to change linearly with weight (W, kg) during the experimental period and was related to infant W as

$$V_b = 0.84 W^{0.82}.$$

Subsequent calculations and their basis are defined in Table 1. (Holland *et al*, 1991; Wells & Davies, 1995).

Table 1 Calculations and assumptions used for calculating breast milk intake

Parameter	*Calculation*	*Origin*
Breast milk intake (*M*)	$M=F_{bm}/0.871$	Breast milk is 87.1% water (Holland *et al*, 1991)
Total water input derived from breast milk (F_m)	$F_m=F_{bm}+0.09M$	The water in breast milk and the water of oxidation of milk solids. These give about 9 g water/100 g breast milk.
Water gained in growth during the experimental period (F_g)	$F_g=(V_{b,14\text{day}}-V_{b,0\text{ day}})/14$	Calculated from the changes in V_b
Total water output from the baby (F_{ob})	$F_{ob}=F_{bb}/0.9915$	A correction for isotopic fractionation assuming that 15% of the infants' water losses were fractionated
Nonoral water intake in the infant (F_a)	$F_a=0.063M$	Water exchange between atmospheric and mainly alveolar water is estimated as 6.3% of milk intake (Wells & Davies, 1995)
Nonmilk oral (supplement) water intake (F_s)	$F_s=F_{ob}-F_m-F_a+F_g$	Water input ($F_s+F_m+F_a$) equals water output plus water from growth ($F_{ob}+F_g$)

Anthropometry

Weight and length of each baby were measured at birth by the medical and nursing students who did the screening interviews. At days 0 and 14 of the deuterium study, the baby was weighed by trained field-workers responsible for the urine and saliva collection. Length was measured at 120 days by field-workers responsible for the follow-up visits. The infants were weighed without clothes using a portable electronic UNICEF scale accurate to 0.1 kg. Length was measured using a standardised anthropometer (AHRTAG baby length measures, London, UK).

Mothers were weighed at day 0 of the deuterium study without shoes but with clothes using the same UNICEF scale, and maternal weight was calculated as the difference between the weight with clothes and the weight of clothes. Maternal height was measured to the nearest millimetre using a locally developed portable stadiometer. Maternal body mass index was calculated from: weight (kg)/length (m)^2.

Maternal body composition

The same dose of 0.5 M deuterium given to the mother for measuring breast milk intake also served to measure maternal total body water. Zero time isotope enrichment (ppm) in the mothers was estimated as the intercept of the fitted maternal isotopic disappearance curve and pool size (N_D, kg) calculated from this dilution of the dose. Pool size was corrected for nonaqueous isotopic exchange to give total body water, fat-free mass was calculated using a hydration coefficient of 73% (International Atomic Energy Agency, 1990), and fat mass as the difference between body weight and fat-free mass that is

$$\text{total body water (kg)} = N_D/1.04$$

$$\text{Fat-free mass (kg)} = \text{total body water}/0.73$$

$$\text{Fat mass (kg)} = \text{body weight} - \text{fat-free mass}$$

Sample

To detect a 100 g/day difference in breast milk intake between exclusively *vs* predominantly breast-fed infants as statistically significant, assuming an s.d. of 130 g/day the study required 27 mother–infant pairs in each group (to a total of 54 infants). These calculations assume a Type I error (α) of 5%, two-tailed, and a Type II error (beta) of 20%, that is, a statistical power of 80%. The s.d. of 130 g/day used in the calculations was based on earlier studies that found a similar or lower s.d. (Lucas *et al*, 1987; Butte *et al*, 1988; Infante *et al*, 1991; Vio *et al*, 1991).

Given the expected rates for discontinuing breast-feeding in the intervention and control groups, only about

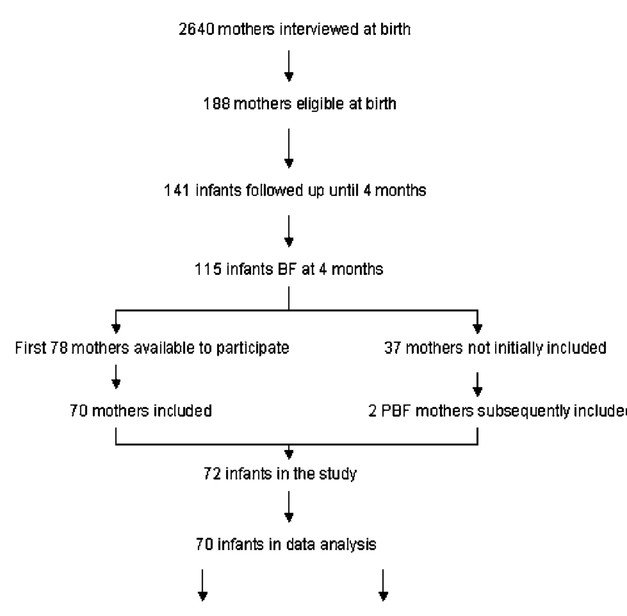

Figure 1 Sampling scheme of mother–infant pairs included in the study.

30% of those recruited would be exclusively or predominantly breast-fed at 4 months. It was decided then that about 180 eligible new-born infants should be recruited.

Figure 1 shows how the final participants were obtained. A total of 2640 mothers were interviewed at birth to provide a sample of 188 mothers eligible for the study. As in the MGRS, the main reason for exclusion was low income. Mothers were next randomised to receive or not receive lactation counselling (Albernaz *et al*, 2003); this was later taken into account in the analysis. During follow-up another 47 mother–infant pairs were excluded from participation: 26 mothers did not want to participate and 21 did not meet eligibility criteria mainly because of the introduction of other milk before 14 days. Of the remaining 141 mother–infant pairs, 115 were still eligible and breast-feeding at 4 months; the first 78 of them were recruited to the breast milk intake study. Eight mothers did not take part because they either refused (six subjects) or were not available at the time. This resulted in 70 mother–infant pairs but the required number of 27 PBF mothers was not reached, so from the remaining mothers that were eligible and breast-feeding at 4 months, only the PBF mothers were asked to participate. This resulted in another two PBF mothers being added and a total of 72 mother–infant pairs participating in the study. At the stage of analysis, two mother–infant pairs were excluded. In one case the baby was breast-fed by both the mother and an aunt, and in the second mother–infant pair the baby stopped breast-feeding during the deuterium study. The results on breast milk intake are therefore based on a total of 70 mother–infant pairs. A criterion of five s.d.'s above or below the mean was used to identify outliers. No subjects had to be excluded for that reason.

As follow-up of the babies was carried out at regular intervals, descriptive data on the eight drop-outs were readily available, and a comparison was made with the mother–infant pairs included.

Calculations of macronutrient intake were restricted to infants receiving liquid foods only in addition to breast milk, that is, infants from the categories EBF, PBF, and BM + FM/CM. From one PBF infant, volumes of intake were not known; therefore, the total number of infants for the calculation of macronutrients was carried out in 61 infants.

Statistical analysis

Differences between feeding patterns were studied using a *t*-test for independent samples, or where $n<5$ Mann–Whitney's *U*-test was used (SPSS software package). Factors considered as potential confounding variables were lactation counselling, maternal age, maternal weight, maternal height, maternal body mass index, maternal fat mass, maternal lean body mass, percentage body fat, maternal education, type of birth (normal or Caesarean), parity, sex of the child, birth weight and length at birth. A factor was considered to be a possible confounder if: (1) its association with the feeding pattern variables had a P-value <0.20, (2) if the association (using Pearson correlation coefficient) with the outcome variables (ie breast milk intake, and intake of non-breast milk water) also had a $P<0.20$, (3) if the factor was known not to be a part of the causal chain between feeding pattern and the outcome variables (Rothman & Greenland, 1998). Multiple linear regression analysis was then used to study the effect of the possible confounder on the outcome variable. A factor was considered as a definite confounder if its inclusion in the equation led to a change of

Table 2 Characteristics of mother–infant pairs by feeding pattern

	EBF (n=35)	*PBF* (n=16)	*PartBF* (n=19)	*Total* (n=70)
Age of the mother (y)	30.0 (5.0)[a]	24.6 (6.1)**	28.5 (5.6)	28.4 (5.8)
Parity	2.1 (2.0)	1.8 (0.9)	2.0 (0.9)	2.0 (1.0)
Years of education of the mother	11.1 (3.3)	11.4 (2.5)	11.2 (3.0)	11.2 (3.0)
Familiar income (reais)	1465.86 (1175.14)	1416.50 (802.45)	1730.58 (993.82)	1526.43 (1046.06)
Familiar income (no. of minimum salaries)	10.8 (8.6)	10.4 (5.9)	12.7 (7.3)	11.2 (7.7)
Mother's height (cm)	159.1 (6.4)	160.3 (5.5)	158.5 (5.3)	159.2 (5.9)
Mother's weight at 4 months (kg)	62.7 (9.3)	58.3 (6.9)	65.7 (11.3)	62.5 (9.7)
Mother's body mass index at 4 months (kg/m^2)	24.8 (3.5)	22.9 (2.7)*	26.2 (4.8)	24.7 (3.9)
Mother's % body fat at 4 months	33.8 (6.1)	30.8 (9.3)	37.3 (7.2)	34.0 (7.5)
Baby's birth weight (kg)	3.2 (0.4)	3.2 (0.3)	3.1 (0.3)	3.2 (0.3)
Baby's length at birth (cm)	48.4 (1.7)	48.8 (2.2)	48.5 (1.5)	48.5 (1.8)
Baby's weight at 4 months (kg)	6.6 (0.9)	6.4 (0.6)	6.5 (0.6)	6.5 (0.7)
Baby's length at 4 months (cm)	63.3 (2,1)	63.5 (2,5)	62.7 (1,8)	63.1 (2,1)
Weight for length (%)	55.7 (29,8)	52.6 (28,1)	59.2 (28,2)	56.0 (28,7)
Sex ratio (male/ female)	19/16	12/4	7/12	38/32
Lactation support (yes/ no)	22/13	7/9	9/10	38/32
Type of birth (normal/Caesarian)	18/17	10/6	7/12	35/35

EBF=exclusively breast-fed; PBF=predominantly breast-fed; PartBF=partially breast-fed.
[a]Mean (s.d.).
*Significantly different from EBF, $P<0.05$; **$P<0.001$.

10% or more in the crude difference between feeding pattern groups.

Results

Classification of feeding pattern

At the time of the deuterium study 35 infants were designated as EBF, 16 as PBF and 19 as PartBF on the basis of the questionnaires. PartBF infants were subdivided into three sub-categories: breast-feeding and receiving formula or cow milk (BF + FM/CM: 11 infants, of those only one infant received cow milk); breast-feeding and receiving complementary foods (BF + CF: three infants), and breast-feeding, receiving complementary foods, and formula or cow milk (BF + FM/CM + CF: five infants).

Description of mother–infant pairs

Table 2 presents characteristics of mother–infant pairs by feeding pattern. Of the infants included in the study, 38 were male and 32 female. The average age of the mothers was 28.4 y. Mothers who were predominantly breast-feeding were significantly younger than exclusively breast-feeding mothers (PBF, 24.6 y and EBF, 30.0 y; $P = 0.001$). On average, parity was 2.0, with 1.8 life-born infants. Generally both the father and the mother had completed second grade schooling (ie, 11 y of education), and family monthly income averaged R$1526 (about USD 970), or 11.2 times the minimum salary for Brazil (R$135). The mean weight of the mothers at 4 months after giving birth was 62.5 kg, and there were no significant differences for PBF and PartBF compared to EBF (EBF *vs* PBF, $P = 0.137$ and EBF *vs* PartBF, $P = 0.294$). In contrast, body mass index was highest in partially breast-feeding mothers, and decreased in exclusively and predominantly breast-feeding mothers (PartBF, 26.2; EBF, 24.8; PBF, 22.9). The difference between EBF and PBF was significant ($P = 0.05$). Similarly, percentage body fat appeared to be highest in partially breast-feeding mothers, although the difference compared to exclusively breast-feeding mothers did not reach significance ($P = 0.06$). The mean birth weight was 3.2 kg and the mean length at birth was 48.5 cm. On average, the mean weight was 6.5 kg, they measured on average 63.2 cm, and they were on the 56th percentile for weight-for-height. In the EBF and PBF feeding category, the male to female ratio was >1, whereas for the PartBF group, the number of males was smaller than the number of female infants.

The eight drop-outs differed from the 70 mother–infant pairs included in that they were older (33.3 *vs* 28.4 y; $P = 0.023$), had more schooling (14.3 *vs* 11.2 y; $P = 0.007$), higher income (18.2 *vs* 11.2 minimum salaries; $P = 0.025$), and all of them had their babies by Caesarean birth ($P = 0.007$). From six of them food intake data were available at 4 months: one infant was EBF, two were PBF, two were PartBF and one was BF + CM/FM.

Confounding variables

Maternal age, maternal body mass index, maternal fat mass and percentage body fat were found to be different ($P < 0.20$) between EBF and PBF, and also associated ($P < 0.20$) with breast milk intake. Multiple linear regression showed that only maternal age and body mass index resulted in a $>10\%$ change in the crude difference in breast milk intake between feeding pattern groups. These confounding variables were included in the subsequent analysis. None of the potential confounding factors were associated with intake of non-breast milk water. For the comparison between EBF and PartBF infants, none of the proposed factors studied fulfilled the criteria for confounding.

Breast milk intake

Means and 95% confidence intervals of intake of breast milk and non-breast milk water (that is the water content of other

Table 3 Intake of breast milk and other liquids by feeding pattern

Reported feeding pattern	N	*Breast milk intake (ml/day)*	P-*value*[a]	*Non-breast milk water intake (ml/day)*	P-*value*	*Total water intake (ml/day)*	P-*value*
EBF	35	806 (753–859)[b,c]	—	10 (0–21)	—	720 (675–766)	—
PBF	16	778 (714–842)[b]	0.590	134 (53–216)	0.005	799 (700–897)	0.091
PartBF	19	603 (473–734)	0.004	395 (225–566)	0.000	921 (754–1087)	0.024
BM+FM/CM	11	585 (357–812)	0.050	536 (267–806)	0.001	1046 (772–1319)	0.025
BM+CF	3	729 (470–987)	0.401	57 (−158 to 273)	0.401	692 (646–738)	0.714
BM+FM/CM+CF	5	569 (378–761)	0.005	288 (251–325)	0.005	784 (625–942)	0.318
All infants	70	746 (696–800)		143 (82–204)		889 (831–947)	

EBF=exclusive breast-feeding; PBF=predominant breast-feeding; PartBF=partial breast-feeding; BM+FM/CM=breast milk+formula or cow's milk; BM+CF=breast milk+complementary foods; BM+FM/CM+CF=breast milk + formula or cow's milk+complementary foods.
[a] *P*-values are compared to EBF.
[b] Values are adjusted for confounders (maternal age, maternal body mass index).
[c] Means and 95% confidence intervals.

liquids or foods), and total intake by food pattern are given in Table 3. Breast milk intake decreased from EBF to PBF to PartBF (EBF, 815 g/day; PBF, 763 g/day; PartBF, 603 g/day). There appeared to be a small difference favouring the EBF group (53 g/day), but this was not statistically significant ($P = 0.26$). Maternal age was a positive confounder, that is, it decreased the difference in breast milk intake between EBF and PBF from 53 to 22 g/day ($P = 0.66$); body mass index was a negative confounder, and augmented the difference to 58 g/day ($P = 0.23$). Adjusted for both confounders, the difference was 28 g/day ($P = 0.59$), with adjusted means of breast milk intake of EBF and PBF of 806 g/day (95% CI 753–859 g/day) and 778 g/day (95% CI 714–842 g/day), respectively. In EBF infants intake of other liquids was 10 g/day, with a 95% confidence interval of 0–21 g/day, that is, not significantly different from zero ($P = 0.08$). Intake of other liquids increased in PBF and PartBF (PBF, 134 g/day; PartBF, 395 g/day). Compared to EBF these differences were highly significant (EBF *vs* PBF, $P = 0.005$ and EBF *vs* PartBF, $P < 0.001$). In addition, total water intake (being a proxy for energy intake) was different between EBF compared to PartBF infants (EBF, 720 ml/day; PartBF, 921 ml/day, $P = 0.024$).

Data were also normalised for body weight of the baby, using breast milk intake (g)/$W^{0.77}$ (kg) (Drewett & Amatayakul, 1999). The overall mean was 176 g/kg$^{0.77}$/day, and was highest in EBF (190 g/kg$^{0.77}$/day). Comparisons between the groups on this basis were not different from the non-normalised data.

We found a positive association between weight for height and weight for age percentiles and breast milk intake ($r = 0.28$, $P = 0.018$, and $r = 0.38$, $P = 0.001$, respectively). However, no associations were found between infant size and intake of non-breast milk liquids.

Pearson's correlation coefficient between the time (months) since introduction of formula or cow milk and breast milk intake was calculated for those infants receiving other milk (BF + FM/CM or BF + FM/CM + CF, $n = 16$). The association was poor ($r = -0.07$, $P = 0.80$).

Figure 2 demonstrates the extent to which breast milk was replaced by intake of other liquids. Consumption of water, tea, juices or complementary foods (that is PBF) had little effect on breast milk intake. In contrast, introduction of formula or cow milk did partially replace breast milk intake (see also Table 3).

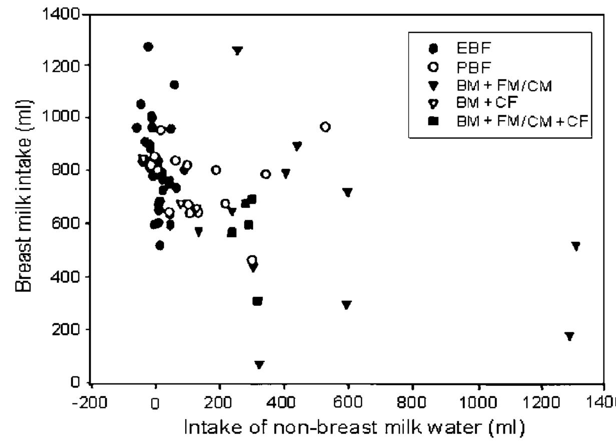

Figure 2 Scatterplot of the association between intake of breast milk and non-breast milk water.

Macronutrient intake

Energy intake of EBF infants was calculated to be 347 kJ (82.6 kcal)/kg/day.

Macronutrient intake (Table 4) was very similar between EBF and PBF. Only protein intake was different between the two groups (1.3 and 1.4 g/kg/day; $P = 0.048$). Energy intake of BM + CM/FM appeared to be higher than in EBF infants (418 *vs*. 347 kJ/kg/day); however, as a result of the large s.d. found in the BM + CM/FM group (135 kJ/kg/day), this difference did not reach statistical significance ($P = 0.11$). Protein and carbohydrate intakes were both significantly higher in BM + CM/FM compared to EBF infants (2.3 *vs* 1.3 g/kg/day, $P = 0.003$; 11.7 *vs* 9.4 g/kg/day, $P = 0.028$).

Comparisons between groups using energy intake normalised for weight$^{0.77}$ (Drewett & Amatayakul, 1999) were not different from the non-normalised data.

Discussion

The issue of replacement of breast milk intake was assessed in PBF or PartBF infants compared to EBF infants. The study was designed to use the same criteria as applied in the MGRS, aiming to assist in interpreting the MGRS results, and to give unique information on nutrient intake of 4-month-old infants growing according to the new growth reference.

Table 4 Macronutrient intake by feeding pattern

	N	*Energy (kJ/kg/day)*	*Protein (g/kg/day)*	*Fat (g/kg/day)*	*Carbohydrate (g/kg/day)*
EBF	35	347 (330–359)[a]	1.3 (1.2–1.4)	4.8 (4.6–5.0)	8.9 (8.6–9.3)
PBF	15	343 (313–372)	1.4 (1.3–1.5)*	4.6 (4.2–5.1)	9.4 (8.7–10.0)
BF+CM/FM	11	418 (326–510)	2.3 (1.7–2.8)**	5.2 (3.9–6.4)	11.7 (9.3–14.0)*

[a]Means and 95% confidence intervals.
*Significantly different from EBF, $P < 0.05$, **$P < 0.005$.

Breast milk intake of EBF infants was on average 806 g/day, and we found no difference between EBF and PBF infants. Breast milk intake of PartBF was reduced to 74% compared to EBF infants. Intake of other liquids was negligible in EBF, and increased in PBF and PartBF infants. Total water intake was highest in PartBF infants, and calculated energy intake in the latter group tended to be 20% higher than in EBF infants.

The study was embedded into a lactation counselling trial (Albernaz *et al*, 2003). Briefly, the outcome of the intervention was that mothers who were not counselled stopped breast-feeding earlier than those who were. However, within the group of breast-fed babies, there was no difference in feeding pattern, nor in breast milk intake between infants from counselled and noncounselled mothers. Therefore, the intervention did not confound our results, and data did not need to be adjusted for participation in the lactation counselling trial.

The number of infants that would be PBF at 4 months was estimated using data from a 1993 birth cohort, and the number of infants recruited at birth was based on this estimation. However, the rate of PBF apparently has reduced in the past few years, resulting in a smaller number of PBF infants in our study than expected. *Posthoc* calculation of statistical power given the numbers actually included and s.d.'s found showed a power of 76% using a one-sided test to detect a difference of 100 g/day in breast milk intake between exclusively *vs* predominantly breast-fed infants. Use of a one-sided test is justified because *a priori* it was expected that intake of other liquids would replace breast milk intake in PBF infants.

Two factors confounded the relation between feeding pattern and breast milk intake: maternal age was a positive confounder, and body mass index a negative one. Although the unadjusted difference between EBF and PBF could have been large enough (7% of daily intake) to have some impact on growth, adjusted for maternal age and body mass index the difference became small (3% of daily intake) and unlikely to affect growth. These findings are in line with those found by Victora *et al* (1998) and recently reviewed by the WHO Working Group on the Growth Reference Protocol (WHO, 2002) showing no difference in growth between EBF and PBF.

Eight mother–infant pairs refused to participate. These mothers appear to have been a select group, as they were higher educated, had higher incomes, were a bit older and all of them had Caesarean birth. Only age of the mother was a confounder in our study; however, as they were evenly distributed among feeding groups, it is unlikely that the results have been biased by their refusal to participate.

On the basis of our findings we make the following conclusions that are supportive of the methodology used in the MGRS: (1) intake of non-breast milk liquids of babies reported to be EBF is negligible, and (2) the difference in breast milk intake between EBF and PBF is small and statistically not significant. This finding suggests that EBF and PBF infants can be pooled for the construction of the growth reference, as their nutrient intake is virtually identical.

One of the limitations of the study concerns its external validity. So that the results could be used for the interpretation of MGRS results, only mothers of high socioeconomic status were included. Another typical characteristic of the participating mothers was that they tended to be overweight (but not obese) (Food and Nutrition Board (FNB), 2002), and this too may have affected breast-feeding behaviour. It is possible that in other populations the introduction of other liquids or foods could have an effect on breast milk intake. An advantage of our stringent inclusion criteria was that important characteristics were the same between groups; for example, birth weight and length, and also weight and length at 4 months, which was invaluable for the comparison between feeding groups.

Our data further show that the introduction of cow milk or formula affects the level of breast milk intake, but it only partly replaces it. Consequently, the total milk intake (breast milk plus cow milk) of BM + CM/FM infants is significantly higher than the total intake of exclusively breast-fed infants. Total energy intake was estimated using a frequency questionnaire for the assessment of intake of non-breast milk liquids. A comparison with the intake of non-breast milk water as obtained from the deuterium-oxide turnover method showed that the FQ tended to underestimate intake. Therefore, our conclusion that total energy intake of BM + CM/FM is 20% higher than of EBF infants is conservative. Indeed, if quantities obtained from the deuterium-oxide turnover method are used to calculate energy intake, the difference between EBF and BM + CM/FM infants becomes as large as 27%. Even though 20% is a considerable difference, it was not statistically significant because of the large variation within the BM + CM/FM group. On the macronutrient level, protein and carbohydrate intake appeared to have contributed most to the increased energy intake, and these differences were significant. It would be tempting to conclude that the BM + CM/FM infants were overfed, but alternatively energy requirements could have been higher in the latter group because of two possible mechanisms: (1) bioavailibility of nutrients from formula or cow milk is less than from breast milk; (2) BM + CM/FM infants may have higher energy requirements compared to EBF infants due to metabolic differences. Although there is substantial evidence that absorption of nutrients from breast milk is higher compared to formula or cow milk (Food and Nutrition Board (FNB), 2002), lower absorption alone could not have explained the large differences in macronutrient and energy intake found. The second mechanism seems to be a more plausible explanation of the differences in intake between EBF and BM + CM/FM found. In the latest review on energy requirements for infants (Butte, 1996), at 4 months an 11% difference between breast- and formula-fed infants was described (Butte, 1996). We calculated the total energy intake of EBF infants to be 347 kJ (83 kcal)/kg/d, which is the same as the suggested modified energy requirements of breast-fed infants by Butte (1996). The total energy intake of BM + FM/CM infants was 418 kJ (100 kcal)/kg/day, which is 20% above energy requirements for breast-fed and 9% above energy requirements for formula-fed infants as given by Butte (1996).

Breast-fed infants appear to have lower requirements than formula-fed infants (Butte, 1996), as has been shown from various studies on energy expenditure using the doubly labelled water method (Davies *et al*, 1990; De Bruin *et al*, 1998; Salazar *et al*, 2000). Butte *et al.* (1990) compared sleeping metabolic rate in breast- and formula-fed babies and found higher values in the latter group. The same author also found differences in sleep organisation between formula or breast-fed infants (Butte *et al*, 1992). These metabolic differences between formula- and breast-fed infants do not necessarily apply to our comparison between subgroups of breast-fed infants, and earlier studies in Pelotas indicate that the surplus of energy is stored, resulting in increased weight gain of PartBF compared to EBF infants during the first year of life (Victora *et al*, 1998). In our study at 4 months we did not find a difference in weight or length between EBF and BM + FM/CM infants, but the latter tended to have a slightly higher weight for length than EBF and PBF infants. It is possible that more eminent differences will occur at a later stage during growth if feeding patterns are maintained. In fact, this different growth pattern between infants who are breast- or bottle-fed is among the primary reasons why WHO has undertaken the Multicentre Growth Reference Study to develop new growth curves based on infants who will be EBF or PBF until 4–6 months of life, and from that age will receive complementary foods in addition to breast milk (WHO, 1994; Dewey *et al*, 1995).

Our data do suggest that intake of BM + FM/CM infants exceeds requirements. In a country in nutritional transition, as is Brazil, with an increasing prevalence of obesity (Mondini & Monteiro, 1997), energy intake above WHO recommendations is not desired, and there is a need for nutrition support directed particularly to mothers who are giving other sources of milk to their babies. Results from our parallel study on the impact of lactation counselling on breast milk intake (E Albernaz, 2000, unpublished results) showed that lactation support increases the relative contribution of breast milk intake in this particular feeding group.

Recently much attention has been given to the possible programming effect of high protein intake in early infancy and the development of obesity later on in life (Parizkova & Rolland-Cachera, 1997). The high protein intake of the BM + FM/CM infants may therefore be reason for concern.

Although only three mothers were categorised as breast-feeding and giving complementary foods, it seems as if the introduction of such foods has little effect on breast milk intake. This seems to be in contrast to earlier findings (Heinig *et al*, 1993), but our numbers are too small to allow any conclusions.

Summarising, breast milk displacement by water, tea or juice is small in infants from mothers from high socio-economic class. Similarly, displacement by other foods also seems to be small. Displacement by other milk is larger, but relatively high volumes of breast milk intake are still maintained. The high energy intake of this feeding category can eventually lead to obesity, a matter of growing concern during childhood in Latin America (De Onis & Blössner, 2000).

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