Breast-milk intake of 9–10-mo-old rural infants given a ready-to-use complementary food in South Kivu, Democratic Republic of Congo

Victor O Owino, Paluku Bahwere, Ghislain Bisimwa, Christine M Mwangi, and Steve Collins

ABSTRACT

Background: Lipid-based ready-to-use foods are currently used for infant feeding, but their potential effect on breast-milk intake is not well documented.

Objective: The objective was to assess the breast-milk intake of 9–10-mo-old infants given either a ready-to-use complementary food (RUCF) paste or a standard corn-soy blend (UNIMIX) porridge in South Kivu, Democratic Republic of Congo.

Design: Infants were randomly assigned at 6 mo of age to receive either RUCF (n = 700) or UNIMIX (n = 700) for 6 mo. Breast-milk intake was measured at 9–10 mo in a subsample of 58 infants (29 from each group). Mothers received a dose of ~30 g deuterium oxide. Predose and postdose saliva samples were collected from both mothers and infants over 2 wk. Breast-milk intake (g/d) was measured from saliva samples by using infrared spectroscopy.

Results: Mean (± SD) breast-milk intake was not significantly (P = 0.69) different between the 2 groups: RUCF (705 ± 236 g/d) and UNIMIX (678 ± 285 g/d). Mean (± SD) nonmilk oral water intakes were 338.3 ± 251.1 and 336.4 ± 227.2 g/d for RUCF and UNIMIX, respectively (P = 0.98).

Conclusions: No differences in breast-milk intake were observed between infants consuming either RUCF or UNIMIX. The deuterium-dose-to-the-mother dilution technique is an affordable technique that we recommend for periodic evaluation of breast-milk intake in resource-poor settings. This trial is registered at controlled-trials.com as ISRCTN20267635. Am J Clin Nutr 2011;93:1300–4.

INTRODUCTION

The global strategy for feeding infants and young children (1) recommends that infants be exclusively breastfed for the first 6 mo after which nutritious complementary foods should be introduced, with breastfeeding continuing through at least the first 2 y of life. Breastfeeding has a positive effect on health throughout infancy and into adolescence. Specific benefits of breastfeeding include protection from infections, reduced risk of obesity, asthma, type 2 diabetes, and reduced atopic and allergic respiratory diseases in later life (2). Breastfeeding for ≥4 mo or longer has been associated with greater scores for fine motor skills and greater adaptability and communication scores at 1–3 y of age (3). Predominant breastfeeding for ≥6 mo is positively associated with academic achievement in children at 10 y of age (4).

Recent reviews (5, 6) showed that lipid-based ready-to-use foods, including ready to use complementary or supplementary foods, may be used to prevent or treat moderate acute malnutrition (MAM) with a positive effect on growth in infants. However, the likely effect on breast-milk intake of feeding infants on highly nutrient-dense ready-to-use foods has not been adequately reported. Such data may help inform breastfeeding promotion and support optimum infant growth and development.

Breast-milk intake can be estimated from measurement of water turnover rates after oral administration of deuterium oxide to the infant’s mother. The deuterium dose-to-the-mother dilution method is a noninvasive, simple, safe, accurate, and reproducible method for measuring breast-milk intake, especially for non-exclusively breastfed infants (7).

The effect of complementary feeding on breast-milk intake in infants by using the deuterium-dose-to-the-mother technique has been assessed in several studies (8–10), but only one study (9) assessed the effect of lipid-based ready-to-use food on breast-milk intake. In the Malawian study, the infants were younger (aged 5–7 mo), the supplementation period was shorter (1 mo), and the lipid-based nutritional supplement was the standard peanut-based ready-to-use therapeutic food with 25% milk powder. However, in the current study, infants were 9–10 mo of age and had been supplemented for 3 to 4 mo at the initiation of the breast-milk study, and the lipid-based nutritional supplement was based on precooked (by extrusion cooking) soybean, maize, and sorghum with only 5% milk powder. More data showing the effect on breast-milk intake of feeding infants with a nutrient-dense ready-to-use food are needed. This study aimed to assess the breast-milk intake of infants 9–10 mo of age consuming either a ready-to-use complementary food (RUCF) or a standard corn-soy blend (UNIMIX) in the context of a large randomized trial assessing the efficacy of RUCF compared with UNIMIX on infant growth. On the basis of findings from Bangladesh (10) showing that increasing the energy density of complementary

1 Address correspondence to VO Owino, Valid Nutrition, PO Box 17191-00100, Nairobi, Kenya. E-mail: victor@validnutrition.org.
2 Supported by Irish Aid, Department of Foreign Affairs, Republic of Ireland.
3 From Valid Nutrition, Nairobi, Kenya (VOO and SC); the Medical and Scientific Center of the Free University of Brussels for its Activities of Cooperation and Centre of Research on Natural Sciences Liwo, Bukavu, Democratic Republic of Congo (GB); the Center for Public Health Research, Kenya Medical Research Institute, Nairobi, Kenya (CMM); Valid International, Oxford, United Kingdom (PB and SC); and the Biostatistic Department of the School of Public Health, Free University of Brussels, Brussels, Belgium (PB).

First published online March 30, 2011; doi: 10.3945/ajcn.110.006544.
foods negatively affects breast-milk intake, we hypothesized that breast-milk intake was likely to be lower in infants consuming highly energy dense RUCF compared with infants consuming standard UNIMIX.

SUBJECTS AND METHODS

Study site

The study was based at Lwiro Pediatric Hospital, Miti Murhesa District, South Kivu Province, Democratic Republic of Congo (DRC). In Eastern DRC, only 24% of infants aged 0–6 mo are exclusively breastfed, and 60% of infants aged 0–2 mo are given water, other liquids, or even solid foods (11, 12). However, this proportion may be increased if community-based volunteers are used to promote exclusive breastfeeding (B Bisimwa, P Nabugobe, N Mitangala, et al, unpublished observations, 2010). By the age of 6–8 mo, 84.9% of all infants will have consumed complementary foods (13). Ethical approval was obtained from the Ethics Committees of the Free University of Brussels, Belgium. Permission to conduct the trial was obtained from the Ministry of Health, South Kivu Province.

Subjects

The breast-milk trial coincided with complete recruitment of all the required number of infants (n = 1400). Mother-infant pairs were selected based on systematic sampling from the database of the large cluster randomized trial assessing the efficacy of RUCF compared with UNIMIX. A data entry clerk who was blind to the identity of the 2 study foods extracted the entire list of all infants who had been recruited into the study by April 2010. All of the infants (n = 420 and 330 in the RUCF and UNIMIX groups, respectively) who had reached the age of 9–10 mo were eligible to be included in the breast-milk intake study. A total of 58 infants were selected (29 from each group) by using a systematic sampling method and ensuring equal representation of both boys and girls. Mothers who had been selected as described above were invited to participate in the breast-milk assessment study because they came to collect their fortnightly RUCF/UNIMIX supply at designated growth-monitoring centers. The selection criteria for infants were as follows: mothers gave informed consent to 1) participate in the breast-milk study, 2) drink the deuterium oxide solution, 3) allow saliva sampling from both herself and the infant for breast-milk intake, and 4) allow for anthropometric measurements on both herself and the infant on days 0 and 14 of the study. In addition, infants were receiving either RUCF or UNIMIX and were not <8.5 mo and not >10.5 mo of age at the time of enrollment into the breast milk intake study. Infants were excluded if the mother did not consent to participate in the breast-milk intake study. In addition, infants who were unwell at the beginning of the breast-milk study were excluded.

Sample size calculation

The sample size was determined on the basis of convenience and limitation on how much deuterium oxide was available. We had enough deuterium oxide stock solution to dose 60 mothers. However, 2 bottles were used for demonstration by the study team, because the mothers requested that the team drink the deuterium first to demonstrate safety. Thus, a total of 58 (29 per group) mother-infant pairs were included in the study. In previous studies, ≥25 mother-infant pairs per group have been adequate to detect mean (±SD) differences of 100 ± 130 g/d in breast-milk intake between groups (8, 14).

Anthropometric measurements

All anthropometric measurements during the breast-milk intake assessment were performed by trained assistants who had already been involved in growth monitoring within the randomized trial. Before the breast-milk, study each of the 10 assistants performed rehearsal measurements on infants until 3 measurements agreed within 0.5 units. Weight, height, and midupper arm circumference (MUAC) for mothers and weight, length, and MUAC for infants were measured on days 0 and 14. Infants’ nude weights and mothers’ weights were measured to the nearest 100 g by using a Salter scale and bathroom scale, respectively. Infants’ recumbent lengths were measured to the nearest 0.1 cm on a portable measuring board. Mothers’ heights were measured by using a stadiometer. Maternal weight and height data were used to calculate body mass index. Head and MUAC were measured to the nearest 0.1 cm by using non-stretchable measuring tape.

Complementary foods

The RUCF used in this study consisted of extrusion cooked maize (Zea mais), soybeans (Glycine max), sorghum (Sorghum bicolor), milk powder, vegetable oil, sugar, and 3% micronutrient powder formulated to provide the recommended daily allowance of all micronutrients for 6- to 12-mo-old infants each day. The energy density of RUCF was 22 kJ/g (5.5 kcal/g). Mothers were instructed to feed the infants 50 g/d in 2 meals without mixing the RUCF with water or other foods. The daily RUCF intake provided 275 kcal. The UNIMIX used consisted of extrusion cooked maize, soybean, and a vitamin and mineral premix. Mothers were instructed to cook in the morning and in the evening 3 spoonfuls of flour (35 g) in boiling water to achieve the suitable consistency of typical porridge with an energy density of 1.1 kcal/mL and then feed the food to the child after it had cooled. The daily intake of UNIMIX (70 g) provided 280 kcal. However, no actual quantification of RUCF or UNIMIX intake was made because it would have required extra time and trained expertise on accurate food intake measurements.

Breast-milk measurement

Breast-milk intake was determined by a deuterium-dose-to-the-mother method as described previously (15). A 30-g dose of deuterium oxide (99.8% purity) was accurately weighed to the nearest 0.001 g. Doses were weighed into sterile Nunc tubes by using a calibrated analytic balance (Sartorius 0.0001 g; Sartorius AG, Goettingen, Germany) at the Kenya Medical Research Institute nutrition laboratory. Preweighed deuterium oxide solutions in sterile tubes were transported by air to the DRC at ambient temperature (25–30°C) within 5 h. The deuterium solutions were immediately held at −20°C for 2 d before use to allow training and subject identification. The frozen solutions were thawed overnight at refrigeration temperature (∼4°C).
On consenting, the mother was asked to rotate a small ball of cotton wool around the mouth until it was completely soaked with saliva. The soaked cotton ball was then squeezed through a 10-mL sterile disposable syringe into a 10-mL polypropylene sterile tube, labeled with the mother’s code, the date and time of sample collection, and weight of deuterium dose. Saliva samples were collected from the infants by a trained technician by rotating the ball of cotton wool below the tongue and around the gum to completely soak. The baseline saliva sampling was done after a fast of ≥30 min. Each mother received an accurately weighed oral dose of ≈30 g deuterium oxide in a clean plastic bottle prepared as described above. The mother was then asked to swallow the deuterium dose using a straw, followed by ≈50 g tap water to rinse down the whole dose.

Postdose saliva samples were collected from the mothers and infants on days 1, 2, 3, 4, 13, and 14 into 10-mL polypropylene sterile tubes. All labeled tubes were separately secured in zip-lock polythene bags and transported in a cool box (0 to −20°C) for storage in a freezer at −20°C while awaiting transportation to the laboratory for analysis. Saliva samples were transported to Kenya for infrared spectroscopy. Saliva samples were analyzed for $^{2}$H enrichment by using Fourier-transformed infrared spectrophotometer at the Kenya Medical Research Institute.

Deuterium enrichment in saliva from the mothers and infants over a 14-d period was measured against a standard in the Fourier-transform infrared spectrophotometer (FTIR 8400 Series; Shimadzu Corporation, Kyoto, Japan). Frozen saliva was thawed before analysis. Deuterium calibration curves to standardize equipment were prepared based on calculated concentration (ppm) compared with measured concentration ($r^2 = 0.999$).

Infant milk intake (g/d) was determined based on 2-compartment model kinetics as described by Coward et al (7). Breast-milk transfer was calculated as described in previous studies (8, 14) 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 infant based on equations and assumptions described in detail in previous studies (8, 14). Breast milk was assumed to be 87.1% water and to have an energy density of 0.67 kcal/g.

**Data analysis**

Data were double entered by using Microsoft Access Software (Microsoft, Redmond, WA). Standard statistical analyses were conducted by using the STATA Data Analysis and Statistical Software (version 10.0; StataCorp, College Station, TX). Analysis of variance was used to compare the infants’ and mothers’ anthropometric characteristics, breast-milk intake, and nonmilk intake. Differences in continuous variables between the RUCF and UNIMIX groups were tested by one-factor analysis of variance.

**RESULTS**

Infant weight, length, MUAC, weight-for-age, length-for-age, and weight-for-length z scores and maternal weight, height, MUAC, and body mass index on day 0 are shown in Table 1. No significant differences in infant weight or length z scores or MUAC were observed between the RUCF and UNIMIX groups at the initiation of the breast-milk intake study. No significant differences in maternal anthropometric indexes were observed between the 2 groups.

Daily breast-milk and water intake partitioning is shown in Table 2. Mean (±SD) breast-milk intake was not significantly (P = 0.69) different between the 2 groups: RUCF (705 ± 236 g/d) and UNIMIX (678 ± 285 g/d). Mean breast-milk intake corrected for infant weight (g · kg$^{-1} · d^{-1}$) was not significantly (P = 0.89) different between groups: RUCF (89.1 ± 30.0 g · kg$^{-1} · d^{-1}$) and UNIMIX (90.4 ± 36.8 g · kg$^{-1} · d^{-1}$). No significant (P = 0.9) difference in nonmilk oral water intake corrected for infant weight was observed between the 2 groups: RUCF (43.2 ± 33.8 g · kg$^{-1} · d^{-1}$) and UNIMIX (44.2 ± 38.1 g · kg$^{-1} · d^{-1}$). No significant correlation was observed between birth weight and breast-milk intake.

**TABLE 1**

<table>
<thead>
<tr>
<th>Infant and maternal characteristics by study group at the beginning of the breast-milk study$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNIMIX (n = 29)</td>
</tr>
<tr>
<td><strong>Infant characteristics</strong></td>
</tr>
<tr>
<td>Weight, day 0 (kg)</td>
</tr>
<tr>
<td>Length (cm)</td>
</tr>
<tr>
<td>Midupper arm circumference (mm)</td>
</tr>
<tr>
<td>Weight-for-age z score</td>
</tr>
<tr>
<td>Length-for-age z score</td>
</tr>
<tr>
<td>Weight-for-height z score</td>
</tr>
<tr>
<td><strong>Maternal characteristics</strong></td>
</tr>
<tr>
<td>Weight, day 0 (kg)</td>
</tr>
<tr>
<td>Height, day 0 (cm)</td>
</tr>
<tr>
<td>Midupper arm circumference, day 0 (mm)</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
</tr>
</tbody>
</table>

$^1$ All values are means ± SDs. RUCF, ready-to-use complementary food; UNIMIX, paste or standard corn-soy blend.

There were no significant differences between the 2 groups in any of the infant or maternal characteristics at the commencement of the breast-milk intake trial.

$^2$ P < 0.05 (one-factor ANOVA and least-squares difference).
DISCUSSION

No difference in breast-milk intake was observed regardless of the study food. The breast-milk intake level in the current study was also slightly higher than the average amount (639 g/d) used for calculating energy needs for infants and young children in developing countries (15). In breastfed children in developing countries, the average energy intakes from breast-milk were 413, 379, and 346 kcal/d at 6–8, 9–11, and 12–23 mo, respectively (15). For the age group in the current study (ie, 9–10 mo), the breast-milk intake is equivalent to 565 g/d assuming an energy density of 67 kcal/100 g for breast milk.

The key limitation in this study was that we were unable to measure breast-milk intake in infants not receiving the study foods. This was so because the main randomized trial in which this study was nested did not include a control group. However, we identified one study (12) that measured breast milk in infants 2–4 mo of age in the same population and in another (8) that assessed the effect of improved complementary foods on breast-milk intake of 9-mo-old Zambian infants. The main differences between the current study and the study by Donnen et al (12) were that 1) the current study used the deuterium oxide technique as opposed to the test-weighing method, 2) older infants were involved in the current study, 3) infants were supplemented with nutrient-dense foods in the current study, and mothers were supplemented with cow milk in the older study. The current study differs from the Zambian trial in several ways, namely that 1) the current study was rural-based as opposed to the Zambian study, which was conducted in a medium-income setting in Lusaka; 2) saliva was collected instead of urine; 3) the analysis was conducted by using infrared spectroscopy as opposed to isotope ratio mass spectrometry. However, the last 2 differences were unlikely to have affected the results because any body fluid may be collected for deuterium enrichment analysis (7), and the 2 analytic techniques have been used with similar results (16).

The overall daily breast-milk intake observed in the current study (691 g/d) is higher than the amount (605 g/d) reported for much younger infants (2–4 mo of age) in the same area (12). It is expected that younger infants may receive more breast milk than those who are already eating other foods, as shown in a study from Malawi (9), where a breast milk intake of 910 g/d was reported for infants aged 5–7 mo. Another study from Bangladesh (17) reported a breast-milk intake of ~800 g/d in 3-mo-old infants. Whereas the low milk intake in the current study may be attributed to the tendency of test weighing to underestimate breast milk, the difference may also be due to better breastfeeding practices after campaigns based on the Global Strategy for Infant and Young Child Feeding, which was published more recently (1). The most recent demographic survey from the DRC (18) reported that the median breastfeeding duration is 21 mo, but it did not indicate the rate of exclusive breastfeeding in mothers with infants <6 mo of age.

The breast-milk intake in the current study is also slightly higher than the amount (639 g/d) reported among 9-mo-old Zambian infants receiving improved complementary foods (8). This slight difference may be explained by the fact that infants in urban settings are likely to be fed more with other foods than are their rural infants. However, both studies show that the provision of nutrient-rich complementary foods do not adversely affect breast-milk intake. In contrast, a study from Bangladesh (10) showed that the increase in the density of complementary foods was associated with a slight reduction in breast-milk intake. However, our results compare well with those from Malawi (9), which showed no differences in the breast-milk intake of infants aged 5–7 mo receiving either a corn-soy blend porridge or a lipid-based nutritional supplement. In the current study, no baseline breast-milk intake was measured at 6 mo of age.

The amount of nonmilk oral fluid intake observed in this study (333 g/d) is consistent with mixed feeding as practiced in this population of infants aged 9–10 mo. The study from Zambia (8) reported a much higher (451 g/d) nonmilk oral fluid intake, and the differences may be attributed to the urban-rural disparity mentioned previously. Much lower levels have been reported for younger infants who consume more breast milk, 168 g/d (9); 40 and 166 g/d for exclusively and nonexclusively breastfed infants, respectively (18).

The results of this study further strengthen campaigns for high-quality complementary foods that can be used to improve the health of infants and young children without interfering with the long-established benefits associated with breastfeeding.

In conclusion, no differences in breast-milk intake were observed between infants consuming either RUCF or UNIMIX. The deuterium-dose-to-the-mother dilution technique is an affordable technique that we recommend for periodic evaluation of breast-milk intake in resource-poor settings.

We thank Insta Products EPZ (Athi River) Ltd for availing their factory for the RUCF production trials, Terry Ajuang for traveling to Lwiro to train enumerators on saliva collection and for transporting samples back to Nairobi, Immaculate Wafula and Benard Otieno (Valid Nutrition, Nairobi) for administrative support, all of the field assistants from Lwiro who helped with the saliva collection, and all of the mothers and infants involved in this study. The authors’ responsibilities were as follows—VOO, PB, and SC: designed and supervised the study, analyzed the data, and wrote the manuscript; GB: supervised the field research and helped write the manuscript; and CMM: analyzed the breast milk in saliva samples and reviewed the manuscript. VOO works for Valid Nutrition, who developed the RUCF. SC is the unpaid Director of Valid Nutrition. PB works for Valid International, a sister company of Valid Nutrition. None of the other authors had a conflict of interest to disclose.

REFERENCES


TABLE 2
Breast-milk and nonmilk oral water intakes for infants by study group and sex1

<table>
<thead>
<tr>
<th></th>
<th>UNIMIX (n = 29)</th>
<th>RUCF (n = 29)</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast-milk intake (g/d)</td>
<td>678 ± 285.4</td>
<td>705 ± 236.1</td>
<td>0.70</td>
</tr>
<tr>
<td>Total water intake (g/d)</td>
<td>1024 ± 247</td>
<td>1096 ± 404</td>
<td>0.41</td>
</tr>
<tr>
<td>Water from breast milk (g/d)</td>
<td>648 ± 246</td>
<td>678 ± 257</td>
<td>0.66</td>
</tr>
<tr>
<td>Nonmilk oral fluid intake (g/d)</td>
<td>336.4 ± 227.2</td>
<td>338.3 ± 251.1</td>
<td>0.98</td>
</tr>
<tr>
<td>Nonoral water intake (g/d)</td>
<td>85 ± 110</td>
<td>69 ± 25</td>
<td>0.46</td>
</tr>
</tbody>
</table>

1 All values are means ± SDs. RUCF, ready-to-use complementary food; UNIMIX, paste or standard corn-soy blend. There were no significant differences between the 2 groups.
2 P < 0.05 (one-factor ANOVA and least-squares difference).


