

Human milk as a source of ascorbic acid: no enhancing effect on iron bioavailability from a traditional complementary food consumed by Bangladeshi infants and young children^{1–3}

Lena Davidsson, Kazi Asif Jamil, Shafiqul Alam Sarker, Christophe Zeder, George Fuchs, and Richard Hurrell

ABSTRACT

Background: Iron bioavailability from traditional complementary foods based on cereals and legumes can be expected to be low unless ascorbic acid-rich foods are incorporated into the diet.

Objective: We evaluated human milk as a source of ascorbic acid for enhancing iron bioavailability from khichuri, a complementary food based on rice and lentils.

Design: Erythrocyte incorporation of stable iron isotopes 14 d after administration was used as a proxy for iron bioavailability. Children aged 8–18 mo ($n = 31$) were breastfed (32–90 mg ascorbic acid/kg human milk) immediately after intake of 4 servings of khichuri labeled with ⁵⁷Fe (test meal B) and were offered water after intake of 4 servings of khichuri labeled with ⁵⁸Fe (test meal A). Test meals were fed twice daily during 4 d in the order of AABBAABB or BBAABBAA.

Results: The mean intakes of human milk and ascorbic acid were 274 g (range: 60–444 g) and 14 mg (range: 4–28 mg, respectively). The mean molar ratio of ascorbic acid to iron was 2.3 (range: 0.7–4.6). The geometric mean iron bioavailability from khichuri fed with or without human milk was 6.2% and 6.5%, respectively ($P = 0.76$, paired Student's t test).

Conclusions: Although human milk contributed significant quantities of ascorbic acid, no significant difference in iron bioavailability was found between khichuri consumed with water and that consumed with human milk. These results indicate either that the molar ratio of ascorbic acid to iron was not sufficiently high to overcome the inhibitory effect of phytic acid in khichuri (30 mg/serving) or that components of human milk modified the influence of ascorbic acid on iron bioavailability. *Am J Clin Nutr* 2004;79:1073–7.

KEY WORDS Complementary feeding, human milk, ascorbic acid, iron bioavailability, infants, stable isotopes

INTRODUCTION

Semisolid foods based on cereals are often some of the first complementary foods to be introduced into the diet of weanling infants (1, 2). The nutritional quality of cereal-based complementary foods is therefore of major importance for ensuring an adequate supply of energy and essential nutrients to rapidly growing infants. Of special concern are the amount and bioavailability of iron in complementary foods, because requirements for absorbed iron during the first year of life are high (3).

Iron bioavailability from cereal products is usually low because of the presence of phytic acid, the major phosphorus storage compound in grains (4, 5). The inhibitory effect of phytic acid has also been shown in infants (6). However, this effect can be overcome by ascorbic acid, a potent enhancer of iron absorption (6, 7). In contrast with industrially produced infant cereals, traditional cereal-based complementary foods that are consumed by infants and young children in developing countries contain virtually no ascorbic acid, and thus these infants and children consume little ascorbic acid unless ascorbic acid-rich foods are incorporated into their diet. Although ascorbic acid-rich foods are readily available in many communities, the intake of fruit and fruit juice by infants and young children might not be encouraged according to traditional feeding practices. For example, we recently observed that 6–18-mo-old children in Côte d'Ivoire consumed monotonous, cereal-based diets and negligible amounts of ascorbic acid from fruit (8). However, although the dietary intake of fruit was virtually nil, an alternative source of ascorbic acid was identified in the Ivorian children's diet: human milk. On the basis of this observation, we hypothesized that iron bioavailability from a traditional complementary food could be enhanced by breastfeeding infants and young children shortly after the intake of semisolid food. The present study was designed to evaluate this potential effect, under realistic conditions, in children living in an area with poor resources.

The aim of this study was to evaluate human milk as a source of ascorbic acid for enhancing iron bioavailability from khichuri, a traditional Bangladeshi complementary food based on rice and lentils. Erythrocyte incorporation of stable iron isotopes 14 d after administration was used as a proxy for iron bioavailability. A crossover design was used to compare iron bioavailability from labeled test meals followed by either breastfeeding or the intake of water in 31 infants and young children.

¹ From the Laboratory for Human Nutrition, Institute of Food Science and Nutrition, Swiss Federal Institute of Technology, Rueschlikon, Switzerland (LD, CZ, and RH), and the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Centre for Health and Population Research, Dhaka, Bangladesh (KAJ, SAS, and GF).

² Supported by the Nestlé Foundation, Lausanne, Switzerland.

³ Reprints not available. Address correspondence to L Davidsson, Laboratory for Human Nutrition, Institute of Food Science and Nutrition, Swiss Federal Institute of Technology, PO Box 474, CH-8803 Rueschlikon, Switzerland. E-mail: lena.davidsson@ilw.agr.ethz.ch.

Received October 6, 2003.

Accepted for publication November 26, 2003.

SUBJECTS AND METHODS

Infants and young children

Breastfed infants and young children (>6 mo old) were recruited from the population in a periurban area, Nandipara, of Dhaka, Bangladesh. All the children had been introduced to complementary foods at the time of recruitment. A spot sample of human milk was expressed and analyzed for ascorbic acid concentration before enrollment.

The study protocol was reviewed and approved by the Ethical Review Committee and the Research Review Committee at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), and by the Ethical Committee at the Swiss Federal Institute of Technology Zurich. Parents were informed about the aims and procedures of the study, and written informed consent was obtained from at least one parent. Mothers and infants were admitted to the metabolic ward at ICDDR,B for the duration of the study (4.5–5 d).

Thirty-two mother-and-child pairs were recruited for the study. Sample size calculations were based on our previous experience with studies of iron bioavailability in infants, in which paired observations were made in 8–10 children per group (6, 7, 9, 10). The sample size was increased ≈ 3 fold because the intake of human milk, and therefore the intake of ascorbic acid, could not be standardized.

Test meals

The test meals consisted of khichuri, a traditional Bangladeshi complementary food based on rice and lentils. Each test meal (50 g “sweet khichuri”) was prepared from 6 g white rice and 3 g lentils (mushur dal), which were cooked in water and mixed with 1.5 g soybean oil and 5 g sugar. All ingredients were purchased in bulk in Dhaka, Bangladesh, and used throughout the study. Batches of sweet khichuri (10 servings) were prepared every day of the study by using a standardized procedure. Before enrollment into the study, the acceptability of sweet khichuri to each child was ensured.

Iron bioavailability

Iron bioavailability was evaluated by using a double-stable-isotope technique (9) based on the incorporation of stable iron isotopes into erythrocytes 14 d after administration of labeled test meals. The study had a crossover design.

Test meal A consisted of sweet khichuri (50 g) labeled with 0.25 mg ^{58}Fe , which was followed by the intake of water. Test meal B consisted of sweet khichuri (50 g) labeled with 0.25 mg ^{57}Fe , which was followed by breastfeeding. The infants and children were randomly assigned to start with test meal A or B. Each test meal was administered 4 times (2 test meals/d on days 1–4) under standardized conditions in the order of AABBAABB or BBAABBAA. Each labeled test meal was prepared immediately before feeding the child by mixing one dose of isotope ($^{58}\text{FeSO}_4$ or $^{57}\text{FeSO}_4$) with a serving of sweet khichuri. The administration of 4 labeled test meals A and 4 labeled test meals B per child was used in this study to minimize the increase in total iron content per test meal because of the relatively high dose of stable iron isotopes compared with native iron in khichuri.

Test meals A were labeled with a total dose of 1.0 mg ^{58}Fe , and test meals B were labeled with a total dose of 1.0 mg ^{57}Fe . $^{58}\text{FeSO}_4$ and $^{57}\text{FeSO}_4$ were prepared from highly enriched ^{58}Fe

and ^{57}Fe metal dissolved in 0.1 mol $\text{H}_2\text{SO}_4/\text{L}$ (9). Individual doses were placed into polytetrafluoroethylene containers, purged with argon, and refrigerated until used. The isotopic composition of the stable-isotope labels was determined by using negative thermal ionization mass spectrometry with FeF_4^- molecular ions (11) and a magnetic sector field mass spectrometer (MAT 262; Finnigan MAT, Bremen, Germany).

All labeled test meals were served ≥ 1.5 h after the last breastfeeding on days 1–4. Immediately after the intake of labeled test meals B, the infants and young children were breastfed to satiety (maximum of 30 min.) The intake of human milk was monitored by weighing the child before and after breastfeeding by using a battery-operated infant scale, which was accurate to 2 g (Seca 727; Medela AG, Baar, Switzerland). Water (100 g) was offered to all children within 30 min after the intake of labeled test meals A. The intake of water was monitored by weighing the cup before and after intake. No additional food or fluid was allowed for 3 h after the intake of each labeled test meal.

Samples of human milk were collected for analysis of ascorbic acid content. Each mother expressed milk immediately after nursing her child (test meal B); 2–3 milk samples were collected from each woman. Sampling was not standardized to a specified time of the day but depended on each child’s feeding habits. The milk samples were mixed with 10% meta phosphoric acid to a final concentration of $\approx 2\%$, protected against light, and frozen until analyzed (8). One sample of human milk was collected in an acid-washed polyethylene container for analysis of iron content.

On day 1, a baseline venous blood sample (2 mL) was drawn for analyses of whole blood hemoglobin concentration and plasma ferritin concentration. A second blood sample (2 mL) was drawn 14 d after the intake of the last test meal (day 18) for analysis of hemoglobin and plasma ferritin and incorporation of stable iron isotopes. Body weight and length were measured at the time of blood sampling. EPI INFO (version 2000; Centers for Disease Control and Prevention, Atlanta) was used to calculate weight-for-height z scores.

Blood analysis

The stable iron isotope composition was measured by thermal ionization mass spectrometry according to the method of Walczyk (11). Whole blood samples were wet-ashed in a nitric acid:hydrogen peroxide mixture by using a microwave system (MLS 1200; MLS, Leutkirch, Switzerland). Iron was separated from the matrix by anion-exchange chromatography, which was followed by a solvent-solvent extraction step into diethylether (9, 12). Isotopic analysis was performed by using negative thermal ionization mass spectrometry with a magnetic sector field mass spectrometer (MAT 262; Finnigan MAT) equipped with a multicollector system for simultaneous ion beam detection (11). Iron separated from the samples was loaded on barium fluoride-coated rhenium filaments of a double-filament ion source together with silver fluoride to promote the formation of negatively charged FeF_4^- ions.

Iron status

Hemoglobin was measured by using the cyanmethemoglobin method (Sigma kit; Sigma, St Louis), and plasma ferritin was measured by using an enzyme-linked immunosorbent assay (Ramco Laboratories, Houston). Commercial quality-control materials from DiaMed (Cressier sur Morat, Switzerland) and Ramco Laboratories were analyzed together with all series of

samples analyzed for hemoglobin and plasma ferritin, respectively.

Calculation of iron bioavailability

Erythrocyte incorporation of stable iron isotopes 14 d after administration was used as a proxy for iron bioavailability. On the basis of the shift in iron isotope ratios in whole blood and the calculated amount of iron circulating in the body, the amounts of ^{57}Fe label and ^{58}Fe label present in blood 14 d after the intake of labeled test meals were calculated according to the method of Walczyk et al (13). Circulating iron was based on blood volume and hemoglobin concentration. Blood volume calculations were based on body weight (14). Because of the high enrichment of the isotopically enriched labels and the low amounts of label incorporated into red blood cells, the data were normalized to correct for mass-dependent isotopic fractionation effects (15).

Food analyses

Samples of freeze-dried sweet khichuri were analyzed for iron content by using flame atomic absorption spectroscopy (SpectrAA 400; Varian, Mulgrave, Australia) after being wet-ashed in a nitric acid:hydrogen peroxide mixture by using a microwave system (MLS 1200; MLS). Standard addition technique was used to minimize matrix effects. The phytic acid content of dry rice and lentils was determined by using an HPLC technique (16).

Human milk analyses

Ascorbic acid in human milk was measured by titration with 2,6-dichlorophenol-indophenol (17). All chemicals (meta phosphoric acid, 2,6-dichlorophenol-indophenol, and ascorbic acid) were purchased from Merck (Darmstadt, Germany). Solutions were prepared fresh for each series of analysis.

Iron content was analyzed by using electrothermal atomic absorption spectroscopy (SpectrAA 400; Varian) with an external calibration curve. Two different sample preparation techniques were used: 1) mineralization by using microwave wet-ashing with a nitric acid:hydrogen peroxide mixture, and 2) dilution with 0.1% Triton X-100 (Fluka, Buchs, Switzerland) and measurement without prior wet-ashing. A certified reference material (nonfat milk powder, SRM 1549; National Institute of Standards and Technology, Washington, DC) was analyzed after wet-ashing.

Statistics

Student's paired *t* test was used to compare iron bioavailability from test meals A and B. Values were logarithmically transformed before statistical analysis. Iron bioavailability data are presented as geometric means \pm 1 SD. All other results are presented as arithmetic means \pm SDs or ranges. Student's paired *t* test was also used to evaluate intakes of human milk and water and to compare the iron content in human milk between the 2 analytic techniques. Spearman's rank correlation was used to evaluate the correlation between iron bioavailability from test meals A and B.

RESULTS

Thirty one infants and young children (16 girls and 15 boys) completed the study. One child vomited after the intake of a labeled test meal and was therefore excluded from the study. The

mean age was 14 mo (range: 8–18 mo). The mean body weight was 8.0 kg (range: 6.6–9.6 kg), and the mean body length was 71.2 cm (range: 65.0–78.6 cm). Wasting (weight-for-height *z* score < -2) was observed in 4 children. The prevalence of anemia and low iron stores was high; 30 children were anemic (hemoglobin concentration < 110 g/L), and 23 and 21 children had a plasma ferritin concentration < 12 $\mu\text{g/L}$ at baseline and on day 18, respectively. All the children were treated with medicinal iron after the study.

Fifteen mother-and-child pairs were recruited during spring and summer 2000. The ascorbic acid content in human milk was 42 ± 10 mg/kg (range: 32–70 mg/kg) in this group. During the second recruitment period (fall and winter 2000–2001; $n = 16$), the ascorbic acid content in human milk was low (< 20 mg/kg), and all 16 mothers were therefore supplemented with 1 g ascorbic acid/d (effervescent tablets, Redoxon; Roche Pharma AG, Reinach, Switzerland). Ascorbic acid was dissolved in water and administered by fieldworkers during 5 consecutive days immediately before the iron bioavailability study. The ascorbic acid content in human milk was 62 ± 13 mg/kg (range: 47–90 mg/kg) in the supplemented mothers at the time of the study.

The total intakes of human milk and ascorbic acid (with test meals B) were 274 ± 85 g (range: 60–444 g) and 14 ± 5 mg (range: 4–28 mg), respectively. The mean molar ratio of ascorbic acid to iron was 2.3 ± 0.8 (range: 0.7–4.6); all the children except 1 had a molar ratio > 1 , 19 children had a molar ratio > 2 , 6 children had a molar ratio > 3 , and 1 child had a molar ratio > 4 . The intake of water (test meals A) was significantly lower (178 ± 59 g; $P < 0.0001$) than the intake of human milk. The iron content in human milk did not differ significantly between the results based on the 2 preparation techniques [0.210 ± 0.120 mg/kg (range: 0.088–0.448 mg/kg) with direct injection compared with 0.221 ± 0.115 mg/kg (range: 0.108–0.506 mg/kg) after wet-ashing]. The iron content in the reference material (SRM 1549; National Institute of Standards and Technology) was 1.71 ± 0.07 $\mu\text{g/g}$ ($n = 6$), whereas the certified value was 1.78 ± 0.10 $\mu\text{g/g}$.

The iron content in sweet khichuri was 0.22 ± 0.02 mg/serving ($n = 9$; freeze-dried weight: 15.2 ± 0.49 g). The mean phytic acid contents in dry rice and lentils were 0.104 and 0.787 g/100 g, respectively. The calculated phytic acid content was 29.8 mg/serving, and the molar ratio of phytic acid to total iron in labeled test meals was 5:1.

Iron bioavailability did not differ significantly between test meals fed with or without human milk; the geometric means ($+1$ SD, -1 SD) with or without human milk were 6.2% (20.8%, 1.8%) and 6.5% (17.6%, 2.4%), respectively ($P = 0.760$). Iron bioavailability from khichuri fed with human milk ranged from 0.64% to 41.4%, and iron bioavailability from khichuri fed without human milk ranged from 0.2% to 27.1%. Iron bioavailability from test meal A was significantly correlated with that from test meal B ($P < 0.01$).

DISCUSSION

The World Health Organization recommends the introduction of complementary foods, in addition to human milk, at 6 mo of age (18), and breastfeeding to satiety or for comfort immediately after the intake of complementary foods can be expected to be common practice in children aged 6–24 mo. The nutritional benefits of breastfeeding infants and young children are well

established, but the present study was designed to evaluate the potential benefit of human milk as a source of ascorbic acid for enhancing iron bioavailability from a traditional cereal-based complementary food. This approach has not been evaluated previously and, if shown to be useful, could have an important effect on iron nutrition during early life. When planning the study, we estimated that the combined intakes of human milk with the 4 labeled test meals of khichuri would be in the range of 200–400 g and would contribute a total amount of 4–12 mg ascorbic acid, based on 20–30 mg ascorbic acid/kg human milk. The molar ratio of ascorbic acid to total iron in the labeled test meals (taking into account that the addition of stable iron isotopes approximately doubled the iron content in the labeled test meals) would be in the range of 0.6:1 to 1.9:1 and would be comparable with the molar ratios (0.9 and 1.66) reported by Derman et al (19) to significantly increase iron absorption from infant cereals in adult women. However, although human milk contributed significant quantities of ascorbic acid in the present study (\bar{x} : 14 mg; range: 4–28 mg), and although the molar ratios of ascorbic acid to iron were in the range in which an enhancing effect would be expected had synthetic ascorbic acid or natural ascorbic acid in plant foods been added to test meals with low phytic acid contents, no significant difference in iron bioavailability was found between khichuri consumed with water and that consumed with human milk.


These results thus indicate either that the molar ratio of ascorbic acid to iron was not sufficiently high to overcome the inhibitory effect of phytic acid in khichuri (30 mg/serving) or that components of human milk modified the influence of ascorbic acid on iron bioavailability. Although a molar ratio of ascorbic acid to iron of 2:1 has been shown to enhance iron bioavailability in adults and older children, a molar ratio of 4:1 is needed to counteract the inhibitory effect of foods high in phytic acid or phenolic compounds [reviewed by Hurrell (20)]. In the present study, only one child had a molar ratio >4 . When the children with a molar ratio >3 ($n = 7$) were evaluated separately, no significant difference in iron bioavailability was observed between the test meals. An alternative explanation might be that ascorbic acid in human milk does not enhance iron bioavailability as efficiently as does synthetic ascorbic acid or natural ascorbic acid in plant foods. Interestingly, a recent study on iron absorption from wheat-based complementary foods in adults (5) reported that cow milk markedly decreases the enhancing effect of dephytinization, both in the presence and the absence of ascorbic acid. Thus, these 2 recent studies show that the presence of milk—human milk or cow milk—influences the enhancing effects of ascorbic acid and dephytinization on iron bioavailability. The mechanisms of the effects clearly need further studies, but note that we reported an inhibitory effect of lactoferrin on iron bioavailability from human milk in infants (10) and that the inhibitory effect of cow milk on iron absorption in adult humans has been related to its high contents of calcium and casein (21, 22).

Although no enhancing effect of ascorbic acid in human milk was found in the present study, the geometric mean iron bioavailability from labeled khichuri was relatively high: 6.2% and 6.5% when fed with or without human milk, respectively. Very few data on iron bioavailability from cereal-based complementary foods in infants have been reported, and comparisons between studies are limited by the large interindividual variation in iron bioavailability. Most of the children in the present study had

low iron stores, and the iron content in the labeled test meals was low; both these factors can be expected to have contributed to the relatively high fractional iron bioavailability. No detailed records of dietary intake are available for the children participating in the present study, but, on the basis of the low native iron content in khichuri (0.22 mg/serving) and the very low content of iron in human milk (\bar{x} : 0.2 mg/kg), daily dietary intake of iron can be assumed to be very low, and the poor iron status of the study population is therefore not surprising.

The concentration of ascorbic acid in mature human milk has been shown to be correlated with maternal intake and plasma ascorbic acid (23). Lower ascorbic acid concentrations in human milk have been reported in African women (24, 25) than in European women, particularly during periods when dietary intake is very low (24). In addition, we recently observed significantly higher ascorbic acid content in human milk collected from European women than in human milk collected from West African women (8). In the present study, the ascorbic acid content in human milk was very low during the second screening study. Although we did not collect information about the dietary habits of the lactating women who participated in the study, the low ascorbic acid content in human milk can be assumed to be due to a low intake of lime juice during the second screening study (S Shamima, R Chanda, and R Ara, personal communication, 2000). Added to rice, lime juice is a major source of ascorbic acid in the diet of women in this community. However, the cost of lime increased significantly between the 2 separate screening studies, which resulted in a reduced intake of ascorbic acid. After supplementation, the mean content of ascorbic acid in human milk was 62 mg/kg (range: 47–90 mg/kg) and was comparable to the value that we previously observed in human milk collected from European women (\bar{x} : 61 mg/kg; range: 33–95 mg/kg) (8).

Although human milk contributed significant quantities of ascorbic acid, no significant difference in iron bioavailability was found between khichuri consumed with water and that consumed with human milk. These results indicate either that the molar ratio of ascorbic acid to iron was not sufficiently high to overcome the inhibitory effect of phytic acid in khichuri or that components of human milk modified the influence of ascorbic acid on iron bioavailability.

The poor iron status of the study children and the very low dietary intake of iron indicated in this study highlight the nutritional vulnerability of this age group. Innovative strategies to improve, at low cost, the nutritional quality of complementary foods consumed by infants and young children in resource-poor areas are clearly and urgently needed. 

We are indebted to the mothers and the children who participated in this study. In addition, we are grateful to Sultana Shamima, Rekha Chanda, and Rawnack Ara of the ICDDR,B for excellent coordination of the study and to Hasan Mahmud for his contribution to the study design. The technical assistance of Marie-Hélène Balsat, Swiss Federal Institute of Technology Zurich, is gratefully acknowledged.

LD designed the study and was responsible for the overall data analysis and the writing of the manuscript. KAJ, SAS, and GF were responsible for the implementation of the study at the ICDDR,B. CZ was responsible for the analysis of the isotopic composition of blood samples and for food analyses at the Swiss Federal Institute of Technology Zurich. All authors reviewed the study protocol and the manuscript. CZ and RH contributed to the preparation of the final manuscript. None of the authors had any conflicts of interest.

REFERENCES

1. Underwood BA, Hofvander Y. Appropriate timing for complementary feeding of the breast-fed infant. *Acta Paediatr Scand Suppl* 1982;294: 1–32.
2. Hervada AR, Newman DR. Weaning: historical perspectives, practical recommendations, and current controversies. *Curr Probl Pediatr* 1992; 22:223–40.
3. Fomon SJ. Nutrition of normal infants. St Louis: Mosby, 1993.
4. Cook JD, Reddy MB, Burri J, Juillerat MA, Hurrell RF. The influence of different cereal grains on iron absorption from infant cereal foods. *Am J Clin Nutr* 1997;65:964–9.
5. Hurrell RF, Reddy MB, Juillerat MA, Cook JD. Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. *Am J Clin Nutr* 2003;77:1213–9.
6. Davidsson L, Galan P, Kastenmayer P, et al. Iron bioavailability studied in infants: the influence of phytic acid and ascorbic acid in infant formulas based on soy isolate. *Pediatr Res* 1994;36:816–22.
7. Davidsson L, Galan P, Cherouvrier F, et al. Iron bioavailability from infant cereals by healthy infants: the effect of dephytinization. *Am J Clin Nutr* 1997;65:916–20.
8. Daneel S. The ascorbic acid content of human milk in relation to iron nutrition. PhD thesis no. 14921. Swiss Federal Institute of Technology, Zurich, Switzerland, 2003.
9. Kastenmayer P, Davidsson L, Galan P, Cherouvrier F, Hercberg S, Hurrell RF. A double stable isotope technique for measuring iron absorption in infants. *Br J Nutr* 1994;71:411–24.
10. Davidsson L, Kastenmayer P, Yuen M, Lönnerdal B, Hurrell RF. Influence of lactoferrin on iron absorption from human milk in infants. *Pediatr Res* 1994;35:117–24.
11. Walczyk T. Iron isotope ratio measurements by negative thermal ionization mass spectrometry. *Int J Mass Spectrom Ion Processes* 1997; 161:217–27.
12. Beer B, Heumann KG. Trace analysis of U, Th and other heavy metals in high purity aluminium with isotope dilution mass spectrometry. *Fresenius J Anal Chem* 1992;343:741–5.
13. Walczyk T, Davidsson L, Zavaleta N, Hurrell RF. Stable isotope labels as a tool to determine iron absorption by Peruvian school children from a breakfast meal. *Fresenius J Anal Chem* 1997;359:445–9.
14. Linderkamp O, Versmold HT, Riegel KP, Betke K. Estimation and prediction of blood volume in infants and children. *Eur J Pediatr* 1977; 125:227–34.
15. Taylor PDP, Maeck R, De Bièvre P. Determination of the absolute isotopic composition and Atomic Weight of a reference sample of natural iron. *Int J Mass Spectrom Ion Processes* 1992;121:111–25.
16. Sandberg A-S, Ahderinne R. HPLC method for determination of inositol tri-, tetra-, and hexaphosphates in foods and intestinal contents. *J Food Sci* 1986;51:547–50.
17. Association of Analytical Communities (AOAC) International. Official methods of analysis. 16th ed. Gaithersburg, MD: AOAC International, 1997:16–7.
18. World Health Organization. Complementary feeding. 2003. Internet: <http://www.who.int/child-adolescent-health/nutrition/complementary.htm> (accessed 1 October 2003).
19. Derman DP, Bothwell TH, MacPhail AP, et al. Importance of ascorbic acid in the absorption of iron from infant foods. *Scand J Haematol* 1980;25:193–201.
20. Hurrell RF. Fortification: overcoming technical and practical barriers. *J Nutr* 2002;132:806S–12S.
21. Hallberg L, Rossander-Hulthen L, Brune M, Gleerup A. Bioavailability in man of iron in human milk and cow's milk in relation to their calcium contents. *Pediatr Res* 1992;31:524–7.
22. Hurrell RF, Lynch SR, Trinidad TP, Dassenko SA, Cook JD. Iron absorption in humans is influenced by bovine milk proteins. *Am J Clin Nutr* 1989;49:546–52.
23. Salmenperä L. Vitamin C nutrition during prolonged lactation: optimal in infants while marginal in some mothers. *Am J Clin Nutr* 1984;40: 1050–6.
24. Bates CJ, Prentice AM, Prentice A, Paul AA, Whitehead RG. Seasonal variation in ascorbic acid status and breast milk ascorbic acid levels in rural Gambian women in relation to dietary intake. *Trans R Soc Trop Med Hyg* 1982;76:341–7.
25. Bates CJ, Prentice AM, Prentice A, Lamb WH, Whitehead RG. The effect of vitamin C supplementation on lactating women in Keneba, a West African rural community. *Int J Vitam Nutr Res* 1983;53:68–76.